

# **The effect of brushing, acid etching and fluoride dentifrice on the surface of human enamel**

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## PREFACE

The present work has been carried out at the Faculty of Dentistry, University of Oslo, during the period 2012-2014. We have chosen to write about the superficial enamel and its appearance in the scanning electron microscope after various clinically relevant treatments (brushing, etching and fluoride application).

The thesis consists of two parts:

- 1) The first part provides basic information concerning the microscopic structure of the enamel, focusing especially on the superficial enamel and organic films covering it. In this part we have also included the effect of acid etching and the pathologic condition erosion. Over the years interest in and emphasis on the prevalence and etiology of erosion has increased.
- 2) The second part consists of our own research project carried out during the summer of 2012 at the Department of Oral Biology. The results are presented as a manuscript entitled: "*The effect of brushing, acid etching and fluoride dentifrice on the surface of human enamel as observed with the scanning electron microscope (SEM)*".

We contacted professor Steinar Risnes in our second year of dental school as our interest for dental hard tissues had increased, and we wanted to get an insight into the research field at the faculty. This resulted in a summer research project funded by *The Faculty of Dentistry in Oslo*.

We are grateful for all the help and support from our supervisors professor Steinar Risnes and associate professor Amer Sehic. In addition we would like to thank Steinar Stølen for valuable help with the laboratory work and with the SEM, Jan Unneberg for help with design, and Alix Young Vik and Lene Hystad Hove for valuable input.

## PART ONE: Introduction

### Physical properties of enamel

Enamel covers the crown of the tooth. The thickness varies between teeth and from individual to individual. It is thinnest at the cervical margin and thickest over the cusps, where it can be about 2,5 mm.

Enamel is the only epithelially derived calcified tissue in mammals and its structure is unique. Enamel is made up of tightly packed crystals of hydroxyapatite. Enamel crystals are long relative to their thickness and highly oriented. They generally extend from the underlying dentin toward the surface of the enamel and are organized into bundles, called prisms. Its structural organization and mineralization give dental enamel its outstanding physical properties, making it the hardest tissue in the body. Its resistance to shearing, impact forces and abrasion is high. It is important that enamel wear progresses slowly because it is not repairable or replaceable. The superficial enamel is less porous, denser and harder than the subsurface enamel.

### Chemical properties of enamel

The mineral in the enamel comprises about 88-90% of the tissue by volume and about 95-96% by weight. Water contributes about 5-10% by volume, and 2% by weight. The remainder consists of organic material.

The chemical formula of the hydroxyapatite unit cell is  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . When fluoride substitutes a hydroxyl group it gives fluorapatite, which has a higher resistance to acid dissolution. The core of the crystal is more soluble than its periphery, probably because it contains more carbonate.

Most of the hydroxyapatite crystals are somewhat hexagonal in cross-section with a width of about 100 nm and a thickness of about 50 nm (see fig. 1). The crystals may be very long and some probably extend from the dentin to the enamel surface.



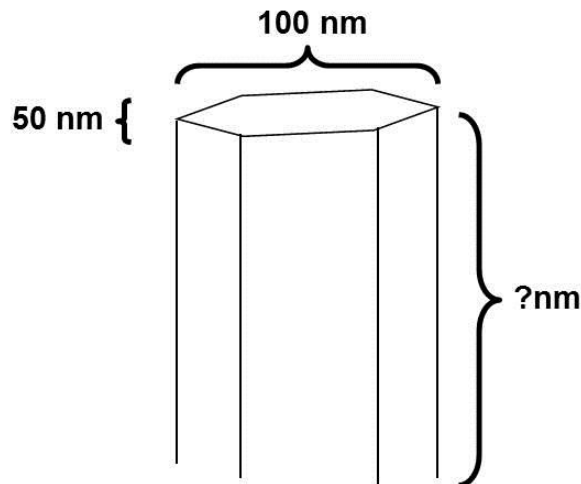


Fig. 1 Schematic representation of an enamel crystal (courtesy of S. Risnes).

## Structure of enamel

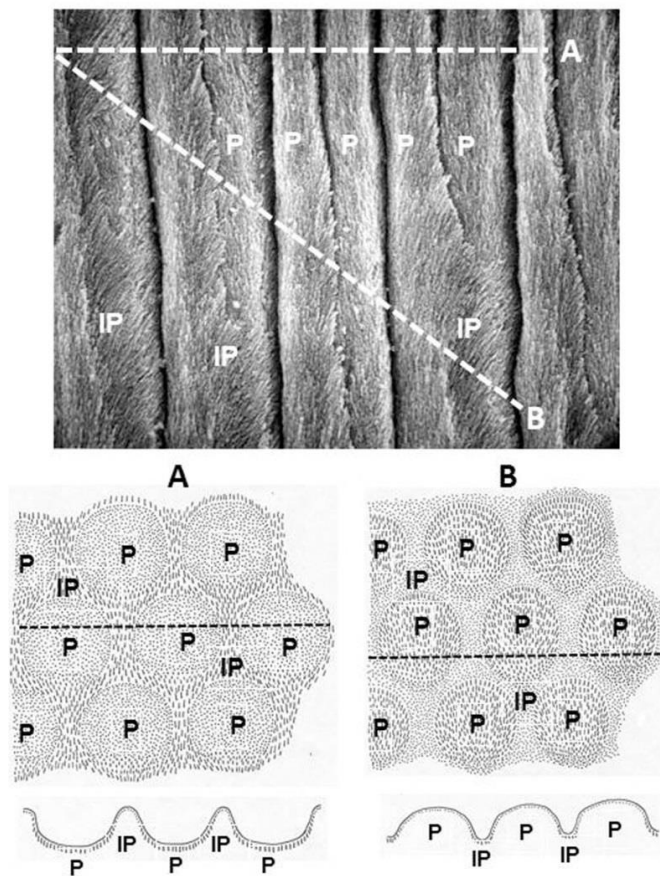
### Prisms and interprism

The enamel prism or rod is the basic structural unit of enamel. The number of prisms equals the number of ameloblasts producing the enamel, each ameloblast producing one prism. The organic matrix constituting the foundation of the prism in which crystals can form and grow is secreted from one aspect of a cellular process protruding from the distal end of the ameloblast, the Tomes' process.

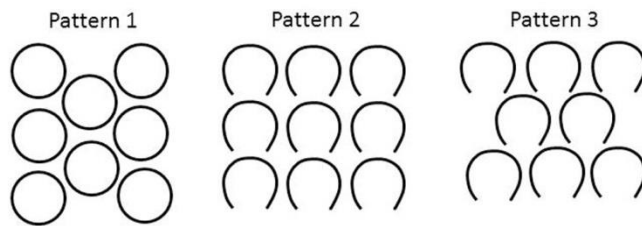
The prisms run from the enamel-dentine junction to the enamel surface and trace out the path pursued by individual ameloblasts during enamel formation. A prism has a diameter of 5-6  $\mu\text{m}$  and consists of a bundle of tightly packed hydroxyapatite crystals oriented parallel with the direction of the prism. Between the prisms is the interprism (see fig. 2). It is also comprised of hydroxyapatite crystals, but the interprism crystals deviate about  $45^\circ$  cervically relative to the crystals of the prisms. Its organic matrix is also secreted by the ameloblasts, not from the Tomes' process, but from the remaining distal surface of the ameloblast surrounding the base of the Tomes' process like a shoulder.

The spatial distribution of prisms and interprism may vary somewhat and consequently three different prism/interprism patterns have been identified. (see figs. 3 and 4). The variable prism/interprism pattern reflects a corresponding

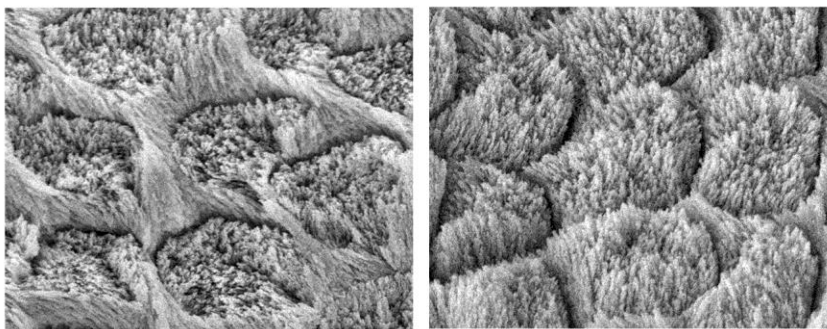
variation in the spatial organization of the ameloblasts and the shape and size of Tomes' processes. It is thought that the crystals tend to be oriented perpendicular to the ameloblast secretory surface. The difference in angle between prism and interprism secretory aspects of the ameloblasts will give a corresponding difference in angle between crystals in prisms and interprism. At the boundary between prisms and interprism, where crystals of different orientation meet, a zone of increased porosity is created, the prism sheath. However, in patterns 2 and 3 this boundary zone lacks on the cervical prism aspect where crystal orientation changes gradually, reflecting a continuous sloping secretory surface of the ameloblast from Tomes' process to shoulder area. In the prism sheath minute amounts of organic material may be accommodated.



*Fig. 2 When acid is applied to an enamel surface that is oriented perpendicularly to the prism crystals (A), the prisms will be preferentially etched. When acid is applied to an enamel surface that is oriented perpendicularly to the interprism crystals (B), the interprism will be preferentially etched. In both instances a rough surface will result (courtesy of S. Risnes).*



*Fig. 3 Schematic representation of the three main prism packing patterns. Pattern 3 is the most prevalent in human enamel, pattern 1 is present in the inner enamel, while pattern 2 is variably present (courtesy of S. Risnes).*



*Fig. 4 Etch patterns of human enamel. In pattern 1 (left) prisms are preferentially etched, in pattern 2 (right) interprism is preferentially etched (courtesy of S. Risnes).*

## The enamel surface

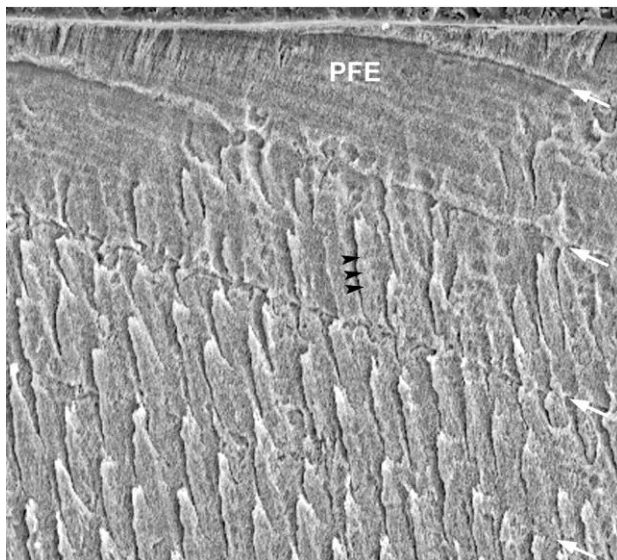
### Superficial enamel

It has been observed that there is a difference in organization and composition between the outermost layer of enamel and the deeper parts of enamel. A review by Speirs (1971) compared “surface” and “subsurface” enamel, where the “surface” enamel was defined as the outermost 100  $\mu\text{m}$ . Numerous reports have described aprismatic enamel in the surface zone (see fig. 5). This is probably due to a loss/retraction of Tomes’ processes toward the end of amelogenesis, leaving a flat secretory aspect with all crystals aligned perpendicular to it and parallel with each other.

The distribution of this prismeless zone does not extend over the entire surface and is most often found in the gingival third of the surface of permanent teeth. It is seen more often in unerupted teeth than erupted. It has been

suggested that this is due to wear and not developmental processes. Prismless enamel is more prominent in temporary than in permanent teeth.

Differences in dissolution rates and hardness of the superficial enamel have been observed between younger and older teeth, and between unerupted and erupted teeth. These differences disappear when the surface is experimentally abraded, indicating that the superficial enamel has distinctive properties and that the cause of this is multifactorial. Once the tooth erupts, it will be covered with pellicle, and biofilm will adhere to the pellicle.



*Fig. 5 Etched facio-lingual section of human tooth showing superficial enamel with Retzius lines (arrows), prisms with cross-striations (arrowheads) and prism-free enamel (PFE). Enamel surface at top (courtesy of S. Risnes).*

### Incremental lines

All hard tissues in the body, i.e. bone, cementum, dentine and enamel, grow in layers. The ameloblasts move when they produce enamel. This movement brings them from the enamel-dentine junction to the surface of the enamel. Although each single ameloblast makes an individual contribution to enamel production, the layered building of enamel is performed by a continuous sheet of ameloblasts, the ameloblastema. The movement of the ameloblastema as a whole is mirrored by the incremental lines of enamel, the Retzius lines (also called striae of Retzius), while the path pursued by each individual ameloblast is

traced out by the prisms. Retzius lines and prism cross-striations are important structural features related to the growth of enamel (see fig. 5).

### *Retzius lines*

Regularly spaced Retzius lines are prominently present in outer and cervical enamel and are suggestive of a rhythm in enamel production. Retzius lines are seen as oblique lines across the prisms and run from the enamel-dentin junction to the surface in longitudinal sections. In horizontal sections they are seen as concentric circles. The lines represent the position of the ameloblast layer at various times during apposition of enamel. The lines that can be observed are representations of three dimensional growth planes in the enamel.

The Retzius lines are seen at intervals between 15-45  $\mu\text{m}$  depending on the location in the enamel. Despite this variation one can find 6-12 cross-striations (supposed daily increments) between each line in any one individual. Therefore, it is believed that the Retzius lines are formed at intervals of approximately one to two weeks. The neonatal line, a prominent Retzius lined formed during birth, indicates that some Retzius lines may be manifestations of stressful situations. The fine, horizontal grooves on the surface of the crown, the perikymata grooves (see figs. 6 and 7), represent the external manifestations of the Retzius lines.

### *Cross striations*

The prism cross-striations are thought to represent a daily rhythm in enamel formation. They are seen as periodic bands across the enamel prisms at intervals of about 4  $\mu\text{m}$ . Cross-striations appear as alternate light and dark bands in acid etched specimens observed in the SEM. In the dark bands there is a reduced crystal concentration.



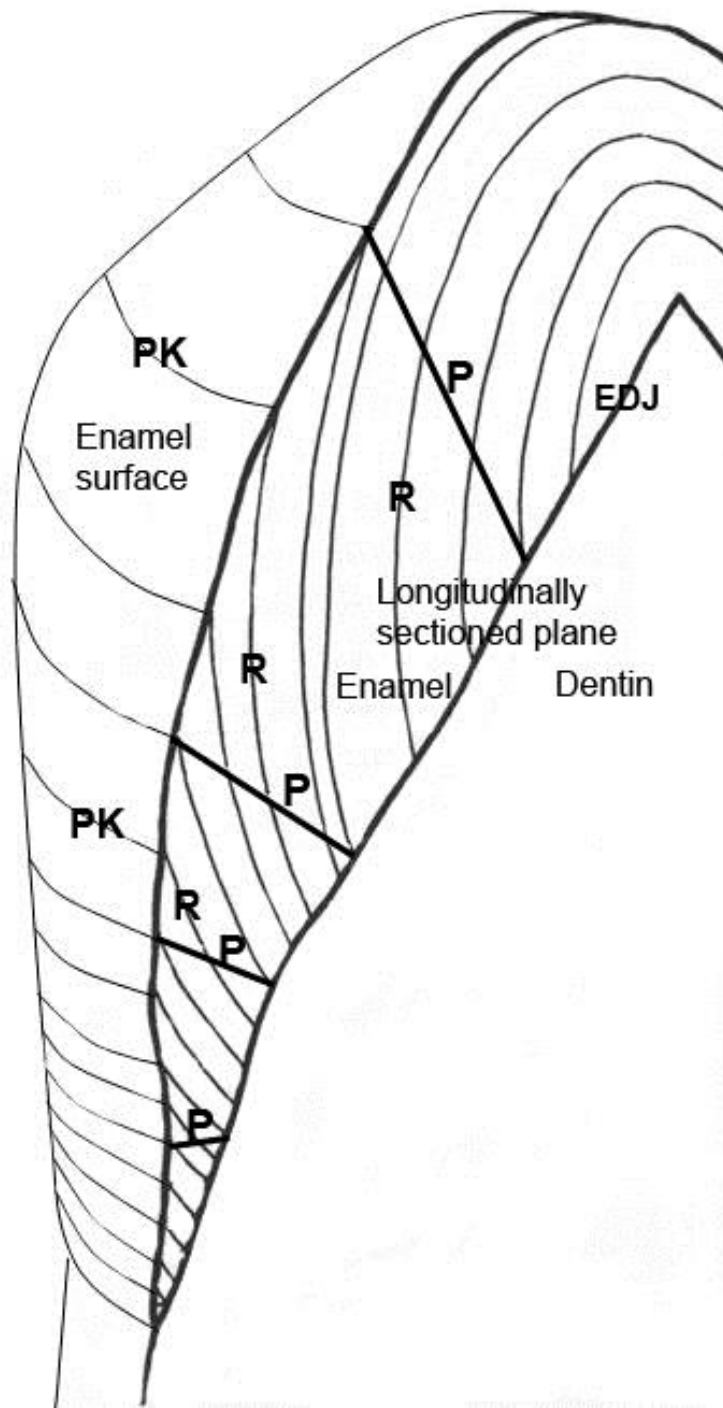


Fig. 6 Schematic representation of facio-lingually hemisectioned tooth showing Retzius lines (R) and their perikymata (PK) representations on the enamel surface. Direction of prisms (P) is indicated at different cervico-occlusal levels. EDJ = enamel-dentin junction (courtesy of S. Risnes).

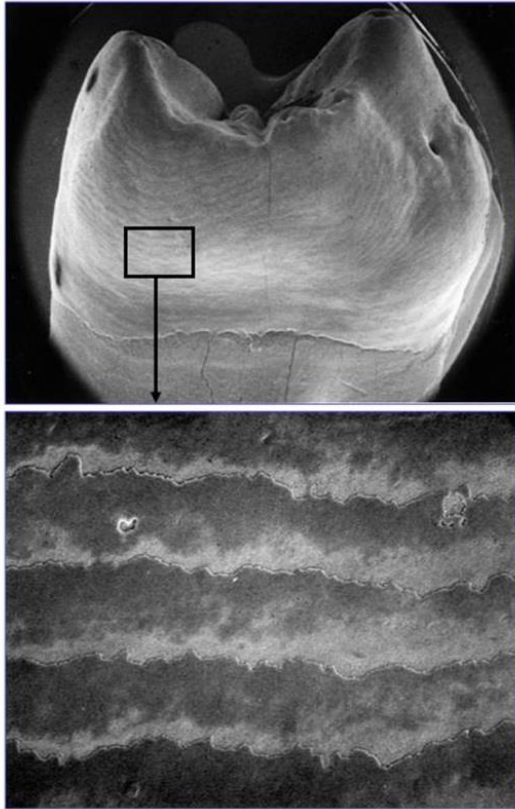


Fig. 7 Perikymata on the surface of a human premolar (courtesy of S. Risnes).

## Organic films on teeth

Saliva is not in direct contact with the tooth since an organic film covers the enamel surface. Through chemical, histological and histochemical methods such a film or membrane has been demonstrated. Organic films have received many names such as “pellicle”, “cuticle” and, when including microorganisms, “biofilm” or “plaque”. “The acquired enamel pellicle” is probably the most acceptable term when describing organic, bacteria-free films. The term “acquired” indicates that the origin of the pellicle is post-eruptive and that it can be regenerated after loss or removal.

Meckel (1965) described and proposed the following classification for organic deposits on enamel:

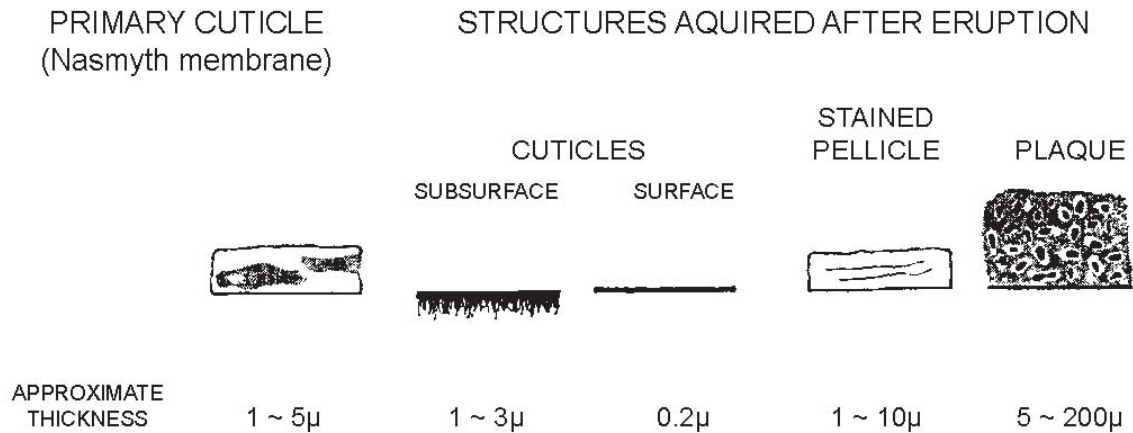


Fig. 8 "Diagrammatic representation of organic films encountered with enamel surfaces." (Modified from Meckel, A.H. (1965))

### The primary cuticle/ Nasmyth's membrane

Nasmyth described the primary cuticle in 1839. It is an organic deposit that comprises the reduced enamel epithelium and the basal lamina. It represents residues of the enamel organ and the dental follicle after tooth development. Most authors agree that it is lost shortly after eruption of the tooth; it has been shown that the membrane is easy to remove with a soft brush and water. Meckel (1965) found in his study an impacted tooth with this layer apparently calcified. It is not known if this is a common occurrence or not.

### Organic films aquired after eruption

The organic films on enamel aquired after eruption were classified by Meckel (1965) as three main types based on their staining and on their appearance in electron micrographs: cuticle, pellicle, and plaque (see fig. 8). The staining reactions of these films were very similar to those of a dried salivary film. Therefore, it was concluded that the films are derived from saliva.

The *surface cuticle* is a very thin and transparent layer and not easily visible on the tooth surface. It is found on sound enamel and also on surfaces that are exposed to considerable friction and wear.

The *subsurface cuticle* is only found in porous areas of slightly damaged surface enamel and never in sound enamel. It consists of a fine mesh of organic material deposited in the surface layer. Because it is embedded in the outermost



layer of enamel, it is only visible after the enamel has been dissolved. It was found that the subsurface cuticle could only be removed by clearing it together with the surface layer of enamel into which it is incorporated.

Above surface and subsurface cuticles Meckel found what he described as a “*stained pellicle*.” This is a bacteria-free, structureless layer of approximately 2µ thickness. Today this film/layer is referred to in the literature as *pellicle*.

In a review by Speirs (1971) it is discussed whether the “subsurface” and “surface” cuticles is simply a continuous part of the acquired pellicle which is more or less integrated in the surface enamel.

Dental *plaque* is a type of biofilm. A biofilm is a microbial community, well organized in a matrix of extracellular material. The dental biofilm develops on the tooth surface. It will not adhere to the enamel surface alone, but the formation begins with attachment to salivary molecules in the pellicle.

### **Composition of pellicle**

The pellicle is a thin, acellular, germ-free film, about 1-10 µm thick, which consists of adsorbed salivary glycoproteins, phosphoproteins, and lipids. It also contains residues of cell walls from dead bacteria and components from the gingival crevicular fluid. Over time the pellicle becomes thicker and it is required for the adherence of microorganisms to the enamel. Some of the proteins and peptides act as receptors for oral bacteria such as *Streptococcus* and *Actinomyces* species. Exposure of previously hidden receptors (cryptitopes) for bacterial attachment may occur when saliva molecules bind to the tooth surface. This is because some receptors undergo conformational changes or enzymically modification by for example neuraminidase.

### **Development of pellicle**

Mechanical brushing with a manual toothbrush does not remove the pellicle layer and it is necessary with detergents from toothpaste or polishing with rubber cups, acid etching or bleaching to remove it. However, it is reformed within minutes. The rate varies between individuals because of differences in salivary flow and

composition. When pellicle is missing, surfaces will be more susceptible to acids and also remineralization. Optimum protection with regard to the pellicle appears to be achieved after pellicle formation of 2 hours or possibly less.

In the normal pH range the enamel surface is charged negatively because of the structure of hydroxyapatite, where phosphate groups dominate close to the surface. A hydration layer is formed because cations, e.g. calcium, are attracted to the surface. Several factors determine the exact composition of this layer, e.g. types of ions present in the saliva, pH and ionic strength. The hydration layer is positive because of the domination of calcium. This implies that it will attract negatively charged salivary macromolecules, such as the ones with acidic side-chains, which is a characteristic component of the acquired pellicle.

### **Function of pellicle**

The pellicle functions as a permeable-selective barrier, e.g. against acids, and restricts the transport of ions in and out of the dental hard tissues. It protects the tooth against chemical and mechanical damage and therefore plays an important modifying role with respect to caries and erosion. The liquid layer within the pellicle has high concentration of calcium and phosphate compared with whole saliva. Therefore the enamel surface will have reduced solubility when covered with pellicle.

The potential role of pellicle composition in determining the composition of the initial plaque microflora has been a topic of considerable interest. Although it has been speculated upon, there is little evidence that variations in the amino acid profile in the pellicle have the potential to modify the different bacteria species adsorption sites.

## Acid etching of enamel surface

In clinical practice acid etching of the enamel surface, or enamel conditioning, is an important procedure. Acid etching is involved when bonding restorative materials, applying fissure sealants or cementing orthodontic brackets to tooth surfaces. There are two desired effects: first, acid etching removes a thin layer of enamel besides plaque and other debris; second, it provides a better bonding surface for adhesive materials and restoratives. The crystals in the exposed surface will dissolve differentially. This will increase roughness and porosity and result in mechanical interlocking of resins to enamel.

The effect of acid etching can be demonstrated by scanning electron microscopy. Different etching patterns can be observed (see fig. 4). Such differences are probably mainly due to differences in the orientation of crystals in prisms/rods and interprism/interrod. Ultrastructural studies indicate that crystals are more susceptible to dissolution at the crystal ends compared to the sides. The most vulnerable crystals are those oriented perpendicular to the enamel surface. (see fig. 2).

## Erosion

Erosion is a chemical process that involves the dissolution of enamel and dentine by acids that are not derived from bacteria. These acids can be of intrinsic or extrinsic origin. Examples of intrinsic origin are vomiting or gastric reflux. Soft drinks, fruit etc. are typical examples of acids of extrinsic origin. The clinical manifestation of erosion is rarely seen isolated, but rather as a combination of erosion, abrasion and attrition. It could be difficult to distinguish between and separate the different conditions due to this very fact. The term erosive wear is now increasingly used and is defined as the combined effect of erosion and mechanical wear on the tooth surface.

According to Mulic et al. (2013) more erosive lesions are registered by Norwegian dentists today compared with 10-15 years ago. The prevalence of

dental erosion among adolescents varies greatly in recent studies and may be difficult to compare due to differences in design and scoring systems of the studies.

### **Clinical appearance**

Erosion of teeth is characterized by complete dissolution of the apatite mineral of enamel and dentin and the lesion appears hard. Erosion typically appears as enamel surfaces that are smooth, silky-glazed and sometimes dull with the absence of perikymata. Often an intact enamel edge can be seen along the gingiva. The advanced lesion shows more pronounced morphology changes like concavities in the enamel and rounding of the cusps in occlusal erosion.

In facial surfaces the width of the erosion lesion is typically larger than the depth of the lesion, whereas in abrasion lesions the depth typically exceeds the width. In erosion, restorations can be observed to be elevated above the level of adjacent enamel or dentin and fracture of the enamel can occur. It is not typical to find erosion and caries on the same surface. This could be explained by the fact that the metabolism of the cariogenic *S. mutans* ceases at a pH below 4,2.

The clinical appearance and severity of erosion also depend on the abrasional and attritional load in the dentition. Also, studies have shown that simultaneous erosion and abrasion resulted in about 50% more wear than alternating erosion and abrasion.

### **Etiology and pathogenesis**

When the dental hard tissues are exposed to an acidic solution that is unsaturated with respect to tooth mineral, the surface will be subject to a layer-by-layer dissolution of the apatite mineral.

When an acidic solution is in contact with tooth substance, it will erode the surface as long as the contact remains. This means that the erosive effect is limited to a short period of time (seconds) just as the solution flushes over the teeth. The solution will also remain on the surface of the tongue between the

papillae. This will enable the tongue to lick away an ultrathin layer of acid-softened hard tissue before the acid is neutralized.

The origin of the acid varies greatly among the affected individuals. Studies show that different acidic drinks cause changes in the pH measured at the dorsum of the tongue. After consuming an acidic drink, there is an immediate pH drop, not lower, however, than the pH of the drink itself. After a minute, the pH is back above 5.5 unless the drink has high buffer capacity and stalls the pH rise with one-two minutes. If the drink contains fermentable carbohydrates, bacteria on the tongue will cause a secondary pH fall after 5 minutes. If this secondary pH fall is of clinical relevance to erosion, is not known. Because the erosion process takes place when the acidic solution flushes over the teeth, it is concluded that in order to prevent erosion it is necessary to rinse the mouth and tongue within the first 30 seconds after drinking.

### **Modifying factors**

Erosive wear is a multifactorial condition. There are different predisposing factors and etiologies (see fig. 9). The different factors will modify the erosive potential in each individual. The interplay between chemical, biological and behavioral factors can explain why some individuals are more affected by the condition than others.

Chemical factors include the pH of the acidic solution, its buffering capacity, mineral content and calcium-chelation properties. Solutions supersaturated with respect to tooth mineral will not dissolve the tooth. If the pH is lower than about 4.0, the solution is under-saturated with respect to hydroxyapatite, and dissolution occurs (see fig. 10). The pH indicates how aggressive the acid is, while the buffering capacity says something about the duration of the challenge before neutralization occurs, either artificially (in vitro studies) or by saliva (in vivo). Organic acids, often used in drinks and foodstuffs, have a high buffering capacity compared to inorganic acids.

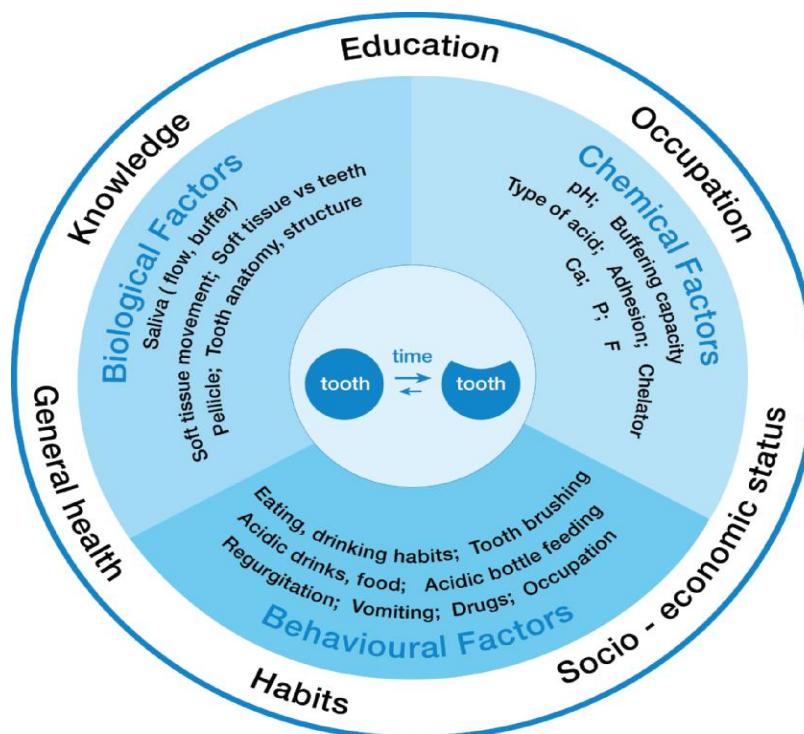


Fig. 9 Adapted from Lussi, A., Hellevig, E., Ganss, C., Jaeggi, T. (2009)

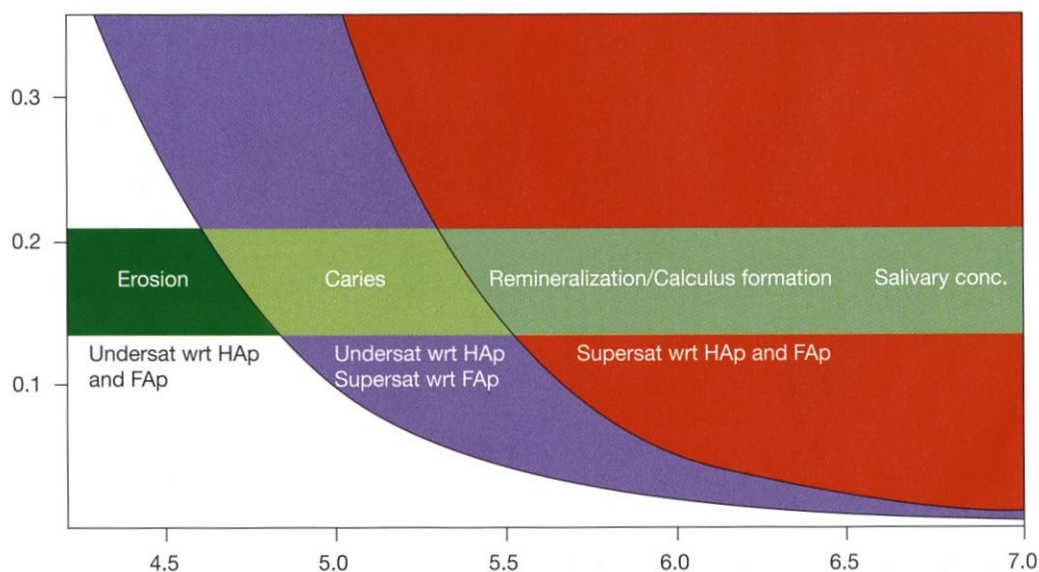


Fig. 10 "Solubility of hydroxyapatite (HAp) and fluorapatite (FAP) as a function of pH in the range 4-6. Above the solubility line for hydroxyapatite, solutions will be supersaturated with respect to (wrt) both HAp and FAP. In saliva, formation of calculus and remineralization of caries lesions may occur. Between the two solubility lines solutions will be undersaturated wrt HAp and saturated wrt FAP. In saliva, HAp tends to dissolve and FAP may form, i.e. a caries lesion may develop. Below the solubility line for FAP, both apatites may dissolve and erosion develop" (Courtesy of Fejerskov, O. & Kidd, E. (2008))

The chelation property of an acid is its ability to bind metal ions. Many organic acids have more than one carboxyl group. Through these groups organic acids can bind calcium and reduce the saturation level of calcium in saliva, or, if strong enough, directly dissolve enamel in order to achieve an equilibrium in the organic acid - saliva - enamel system with respect to calcium. One study shows that citrate, at common fruit juice concentrations, can complex up to 32% of the calcium in saliva. This means that the saliva's super-saturation with respect to calcium will be reduced and thus increase the driving forces for dissolution of tooth minerals.

Saliva acts as a protective factor against erosion. It provides proteins for the organization of pellicle and this will give benefits as pellicle (and also plaque) may act as a diffusion barrier for acids. During intake or contact with acidic solutions the saliva production in the salivary glands is increased. This helps both in diluting and removing acid as it flushes over the teeth. Another important protective mechanism is the buffering capacity of saliva. Stimulated saliva contains high amounts of bicarbonate and, thus, has a high buffer capacity that will help in neutralizing the acid.

### **Use of fluoride in dental erosion**

Different ways of preventing erosion have been considered in other studies. The use of fluoride has been debated over the years. Research on fluoride's protective effect has shown a wide range of results. Some studies have reported the presence of a protective effect while others have not. Various reasons for this have been discussed. Sorvari et al. (1994) in their study of Duraphat varnish suggested that the varnish layer itself may act as a barrier against erosion. The results also vary dependent on the type of fluoride solution used. It has been found that an acidified fluor gel might be superior to a gel with a neutral formulation. Hove et al. (2007, 2008) documented a protective effect against erosion by using  $\text{TiF}_4$  solution. This solution will, according to the authors, create



a complex between  $\text{TiF}_4$  and proteins on the enamel surface and form a "glaze" which may function as an acid-resistant layer.

Fluoride has anticariogenic properties and may possibly, through similar acid-resistant mechanisms, enhance hard tooth tissues resistance against dental erosion. Fluoride seems to be able to make an eroded enamel surface hard again and therefore be able to improve its abrasion resistance. Ganss et al. (2001) have in their study concluded that there was a significant reduction in the progression of erosion in enamel using intensive fluoridation after 5 days, but there was a more pronounced effect on dentine.

Fluoride is mostly retained as a  $\text{CaF}_2$ -like material by the application of toothpaste, gel or mouthwash. This has been shown to last on tooth surfaces for weeks or months under neutral conditions. The mechanism of action of fluoride in the prevention of dental erosion is not well known. It can be speculated that in the case of enamel, the  $\text{CaF}_2$ -like layer can be dissolved under acid attack before the underlying enamel is attacked. In spite of the varying results of fluoride's effect against erosion, it is at present in accordance with good clinical practice to recommend fluoride as part of the treatment.



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Lecture by Alex Young Vik 6th semester 2012

Lecture by Aida G. Mulic 16.02.12

<http://www.dentalcare.com/en-US/dental-education/continuing-education/ce374/ce374.aspx?ModuleName=coursecontent&PartID=7&SectionID=-1>

## **PART TWO: The effect of brushing, acid etching and fluoride dentifrice on the surface of human enamel as observed with the scanning electron microscope (SEM)**

### **Aim of study**

The aim of the present in vitro experiment was to examine the effects on the enamel surface of various combinations of acidic and abrasive challenges. The method of examination was scanning electron microscopy (SEM).

### **Material and methods**

Human maxillary premolars and third molars were obtained from the collection of extracted teeth at the Department of Oral Biology, Faculty of Dentistry, University of Oslo. After extraction the teeth had been stored for a long period in 70 % alcohol, then left to dry. The criteria for selection of teeth to be included in the study were intact and clean facial and lingual aspects as judged by macroscopic observation. The teeth were fixed to a holder by dental wax without compromising the facial and lingual aspects, placed in a cutting device, and cut mesio-distally in facial and lingual halves with a turbine-driven diamond disk cooled by water (1). Two thirds of the root was also removed by cutting. The facial and lingual aspects were sprayed with dishwasher detergent (Zalo, Lilleborg, Norway), brushed with a toothbrush for 1 minute and rinsed under running tap water for 30 seconds.

The material was divided into 12 groups (A-L) according to procedure (Table 1). There were 48 teeth in total: four teeth (eight halves) in each group, two premolars (four halves) and two molars (four halves), the two halves of each tooth included in the same group.

The teeth of group A did not receive any additional treatment after the general cleaning procedure. Groups B-D were subjected to one of the brushing procedures described in table 1, group E was only etched, while groups F-L were

subjected to combinations of etching and brushing. The purpose of these various preparations was to investigate the effects of toothbrushing, acid, and the use of toothpaste with (Solidox Total beskyttelse, Lilleborg, Norway) and without sodium fluoride (Solidox, Lilleborg, Norway) on the enamel surface.

All teeth were placed in a heating cabinet (temperature 35 °C) before they were glued to aluminium stubs with facial and lingual aspects facing upwards. After sputter coating with a layer of platina, approximately 300 Å in thickness, the surfaces were examined in a scanning electron microscope (Philips XL30 ESEM, Philips, FEI, Netherlands) operated at 12-16 kV. In general, four pictures were obtained of each specimen, with magnifications of X12-20, X100, X1000, and X5000. From selected areas pictures with a magnification of X10000 were obtained.

After having observed a membrane-like structure on the enamel surface of molars in the SEM, about 1-2 mm thick longitudinal facio-lingual sections were cut from five of the molar specimens. The cut surfaces were observed under a dissection microscope. One section from each tooth was further polished with 1200 grit waterproof silicon carbide paper (3M, St. Paul, MN, USA) and 1.0 µm particle size alumina powder (Buehler Micropolish, Buehler, Lake Bluff, IL, USA), and then rinsed under running tap water. The sections were air-dried at room temperature, acid etched with 0.5% nitric acid for one minute, rinsed with distilled water for 30 seconds, air-dried and placed on stubs. They were coated with platina and observed in the SEM.

The REA value of the toothpastes could not be obtained. Three toothbrushes with medium bristle stiffness (Jordan, Norway) were used, one for the teeth that were only brushed, and the other two for each of the groups that were brushed with toothpaste, either with or without sodium fluoride. The nitric acid was renewed for each group that was etched with acid.

Tabel 1. Material and methods. Procedures applied to each group of teeth. Numbers indicate order of procedures.

Procedures	Groups											
	A	B	C	D	E	F	G	H	I	J	K	L
Basic preparation*	1	1	1	1	1	1	1	1	1	1	1	1
Etch 1 min 0,5% HNO <sub>3</sub> ** + rinse					2	3	3	3	2	2	2	2
Brush 1 min + rinse									3			
Brush tp (no F) 1 min + rinse		2				2				3		
Brush tp (F) 1 min + rinse			2				2				3	
Brush tp (F) 1 min, wait 30 min + rinse				2				2				3
Remove excess water + drying	2	3	3	3	3	4	4	4	4	4	4	4

\*Clean with toothbrush and soap 1 min., rinse under running tap water 30 sec, remove excess water. \*\*Before etching excess water from previous rinsing was removed. tp = toothpaste, F = fluoride



Fig.1. Shows a selection of sputter-coated facial and lingual tooth halves and facio-lingual sections mounted on aluminum stubs.

## Results

Macroscopically the enamel surface of both premolars and molars appeared clean after the basic brushing and rinsing procedure. However, when observing the teeth in the scanning electron microscope, it became evident that the enamel surface on molars generally was covered by a relatively thick coating which profoundly affected the effects of the procedures. Hence, premolars and molars will be described separately.

### Premolars (Figs. 2-13)

The coating generally present on molars was generally absent on premolars. Elements of a coating were only observed on two out of 24 premolars (Figs. 4a, 11a,d,e).

#### *Group A (basic cleaning + rinsing, Fig. 1)*

Perikymata were variably apparent (Fig. 2a,d,g,j), probably primarily dependent on the degree of surface wear in the mouth before extraction. Prism profile ends were also variably evident (Fig. 2b,e,f,h,i). Scratches were especially seen on the most worn surfaces (Fig. 2g,h,j,k).

*Groups B (basic cleaning + brushing w/paste + rinsing, Fig. 3), C (basic cleaning + brushing w/Fpaste + rinsing, Fig. 4), and D (basic cleaning + brushing w/Fpaste + waiting 30 min + rinsing, Fig. 5)*

Additional brushing with toothpaste did not have any striking additional effect on the enamel surface compared to the surfaces seen in group A. However, it may seem that prism profile ends more generally presented themselves as shallow depressions (Figs. 3b,c,e,f,h,k, 4b,h,i,k,l, 5b,e,f). Also, brushing with toothpaste left a grainy-fluffy material on the surface which was not completely removed by rinsing (Figs. 3f, 4e,f,h,i), especially when leaving the paste on the surface for 30 minutes before rinsing (Fig. 5b,c,e,f,h,i,k,l). The material was unevenly distributed and its elements varied in size and shape. On some enamel surfaces a deposit of relatively evenly distributed and sized spheres with a diameter of about 0.1  $\mu\text{m}$

was seen (Figs. 3c,f,l, 4l, 5f,i,l). There were no obvious differences between the three toothpaste groups.

*Group E (basic cleaning + etching, Fig. 6)*

Etching the surface with acid yielded a distinct etch pattern with differential etching of prisms and interprism, with marked topography and prism sheaths. Areas of prism-free enamel was also encountered (Fig. 6b,e,h).

*Groups F (basic cleaning + brushing w/paste + rinsing + etching, Fig. 7), G (basic cleaning + brushing w/Fpaste + rinsing + etching, Fig. 8), and H (basic cleaning + brushing w/Fpaste + waiting 30 min + rinsing + etching, Fig. 9)*

When brushing with toothpaste was followed by etching, very little of the grainy-fluffy remnants of the toothpaste remained on etched enamel surfaces (Figs. 7-9). The effect of etching on the superficial enamel was varied concerning distribution/pattern, distinctness, and shape of prisms (Figs. 7-9 b,e,h,k). This variation seemed more related to the inherent structure of the superficial enamel than to the differences in methodological procedures. Hence, the effect of acid etching did not seem to be moderated to any appreciable extent by preceding brushing with toothpaste (compare with Fig. 6 which shows etching without previous brushing with toothpaste).

*Groups I (basic cleaning + etching + brushing + rinsing, Fig. 10), J (basic cleaning + etching + brushing w/paste + rinsing, Fig. 11), K (basic cleaning + etching + brushing w/Fpaste + rinsing, Fig. 12), and L (basic cleaning + etching + brushing w/Fpaste + waiting 30 min + rinsing, Fig. 13)*

Brushing after etching invariably smoothed and blurred the etch pattern, rendering topography and prism boundaries much less obvious (Figs. 10-13). No appreciable differences in smoothing of the etch pattern were observed at medium magnification between brushing with no paste (Fig. 10b,e,h,k), brushing with nonfluoride paste (Fig. 11b,e,h,k), and brushing with fluoride paste (Fig. 12b,e,h,k). Leaving the fluoride paste on the surface for 30 minutes before

rinsing did not seem to give any additional effect at this magnification (Fig. 13b,e,h,k). Also at higher magnification it was difficult to discern a difference between teeth brushed with fluoride paste (Groups K and L, Fig. 12c,f,i,l and Fig. 13c,f,i,l) and teeth brushed with no paste (Group I, Fig. 10c,f,i,l) or nonfluoride paste (Group J, Fig. 11c,f,i,l). However, it may seem that the crystals in fluoride-brushed teeth were generally more distinct and generally more tightly packed than in teeth that had not been subjected to fluoride. Brushing without toothpaste did not leave any material on the surface (Fig. 10), while brushing with toothpaste could leave small amounts, especially after leaving the fluoride paste on the surface for 30 minutes before rinsing (Fig. 13e,f,h,i).

### **Molars** (Figs. 14-20)

The enamel on molars was generally, but to a variable extent, covered with a coating of unknown character. It revealed its presence by its absence in some areas (Figs. 14a,c,f,g, 15b,e,f, 16a,e,f,g, 17a,b,c,f,g, 18a,b,e,f,g, 19a,b,f,g) and by hiding and obstructing the visibility of prism ends. The coating appeared generally structureless but for occasional bumps, holes, and depressions, and had a smooth to velvety texture (Figs. 14d,e, 16c,d, 18c,d).

Although the coating was not readily removed by brushing with standard medium toothbrushes, with or without toothpaste, brushing seemed to sever the coating in a patchy manner (Figs. 14a,c,f,g, 15b,e,f, 16a,e,f, 17a,b,f,g, 18a,e, 19a,b,f,g). Where the coating was missing or had been removed, the enamel surface proper was readily visible (Figs. 14g,h, 15f,g). When brushing was followed by etching, the coating became more conspicuous because of the contrast created by the etching effect on the exposed enamel surfaces (Figs. 16f,g, 17b,c,g). The coating itself seemed unaffected by the etching (Figs. 16b-d,f,g, 17b,c,g, 18g). It appeared that the coating protects the enamel surface from the etching effect of acid. This is demonstrated in Fig. 19b,c,g,h where three surfaces are visible: coating, intact enamel surface, and etched enamel. A likely explanation is that the etched enamel areas had no coating during etching, while the unetched enamel areas were coated and thus protected during etching, but



were relieved of their coating during the subsequent brushing. Brushing with toothpaste left a grainy-fluffy material on the surface, more on the coating than on enamel surfaces (Figs. 14h-k, 15c,g-j, 16g, 17c-e, 18g-i).

On longitudinally sectioned, ground and etched molars the surface coating was clearly evident (Fig. 20). Its thickness varied somewhat, but was generally in the order of about 10-40  $\mu\text{m}$ . Its internal structure could be observed where it was fractured, but no distinct characteristic structural elements could be identified (Fig. 20b-c,f-g). Generally, there was a cleft between the coating and the enamel surface (Fig. 20c,d). The coating appeared to be continuous with material observed in an enamel cleft (Fig. 20f). The material in the cleft exhibited impressions of longitudinally oriented prisms (Fig. 20h). On the occlusal aspect an additional coating layer was observed (Fig. 20a,b,e-g). It was somewhat thicker than the general coating and seemed to contain some structural element oriented vertically or obliquely to the surface (Fig. 20g).

### Legends to figures 2-20

Figures 2-13. Scanning electron micrographs of groups A-L premolars. a-c) Facial aspect and d-c) lingula aspect of same tooth, crown shown in insets. g-i) Facial aspect and j-l) lingual aspect of same tooth, crown shown in insets. Some aspects show severed enamel probably inflicted during extraction. C = coating, EE = etched enamel, UE = unetched enamel, IP = interprism, P = prism, PFE = prismfree enamel.

Figure 2. Group A. a,d) Distinct perikymata, g) faint perikymata, j) worn surface without perikymata. b) Prism ends appear as faint, rounded elevations, e) variably expressed prism ends, h,i) arcade shaped prism ends.

Figure 3. Group B. a,d,g,j) Distinct perikymata. b,c,e,f,h,k) Variably expressed prism ends. f) Toothpaste material left on enamel surface. l) Deposit of fine spherical globules.

Figure 4. Group C. a,g,j) Distinct perikymata, d) worn and partly coated surface without perikymata. b,h,i,k,l) Variably expressed prism ends. b,c,e,f,h,i) Toothpaste material left on enamel surface.

Figure 5. Group D. a,d,g) Variably distinct perikymata, j) worn surface without perikymata. b,c,e) Variably expressed prism ends. b,c,e,f,h,i,k,l) Toothpaste material left on enamel surface. i,l) Deposit of fine spherical globules.

Figures 6-9. Groups E-H. Etched enamel surfaces with aspects showing horizontal zones indicating perikymata pattern, and with exposed prisms, interprism and areas with prismfree enamel.

Figures 10-13. Groups I-L. Etching effect blurred and smoothed by brushing. Perikymata pattern and prisms are variably visible and may be difficult to identify.

Figures 14-19. Scanning electron micrographs of groups A-L molars. Facial or lingual aspects. All molars exhibit a coating on the enamel surface. Perikymata pattern may be visible, irrespective of presence or absence of coating. C = coating, EE = etched enamel, ES = enamel surface, UE = unetched enamel, IP = interprism, P = prism, PFE = prismfree enamel.

Figure 14. Groups A and B. b,c,d,e,g,i,k) Surfaces with coating. c,g) Coating is partly missing, exposing enamel surface. h,j) Uncoated enamel surface. j) Enamel surface with spherical globules. i,k) Fluffy-grainy material on coating.

Figure 15. Groups C and D. b,c,d,f,h,j) Surfaces with coating. b,c) Coating is partly missing, exposing enamel surface. c,d) indicates surface of uncertain character. c,d,h,j) Fluffy-grainy material on coating. g,j) Only small amounts of material on enamel surface.

Figure 16. Groups E and F. b,c,d) Surfaces with coating. f,g) Areas of etched enamel where coating is missing. h) Etched enamel. d) Spherical globules on coating. g) Fluffy-grainy material on coating.

Figure 17. Groups G and H. b,c,g) Areas of etched enamel where coating is missing. d) Coated surface with fluffy-grainy material. e,h,i) Etched enamel.

Figure 18. Groups I and J. b,f,g) Coated surfaces with enamel areas lacking coating. c,d) Surfaces with coating. g,h) Fluffy-grainy material on coating. i) Enamel surface (uncertain if it is unetched or etched and blurred by brushing).

Figure 19. Groups K and L. b,c,g,h) Surface with coating, enamel surface/unetched enamel, and etched enamel. d,i) Etched enamel. e) Coating with fluffy-grainy material and enamel surface/unetched enamel. j) Enamel surface/unetched enamel. k) Coating with fluffy-grainy material.

Figure 20. Scanning electron micrographs of faciolingually sectioned, ground and etched molars. a,e) Facial cusp part. b,f,g) Two layers of coating are present occlusally, an inner one covering the whole crown and an outer one restricted to the occlusal aspect. c,d) Between the general coating and the enamel there often seemed to be a cleft. f,h) The inner, general coating was continuous with material in an enamel cleft or lamella. The material exhibited impressions of the longitudinally oriented prisms in the wall of the cleft. Arrow indicates prism direction. C = coating, CM = cleft material, D = dentin, E = enamel, ES = enamel surface, OC = occlusal coating.

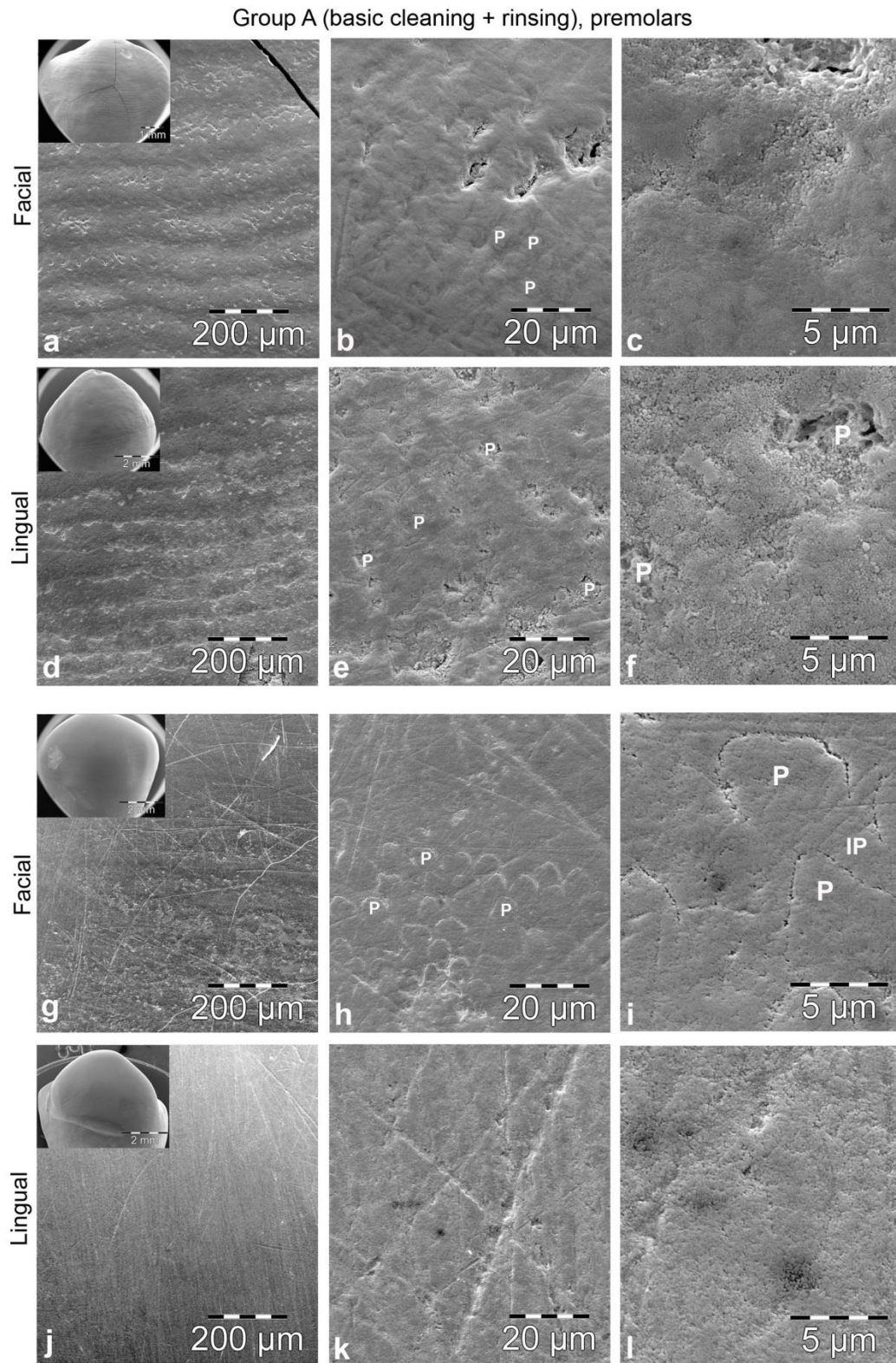


Figure 2



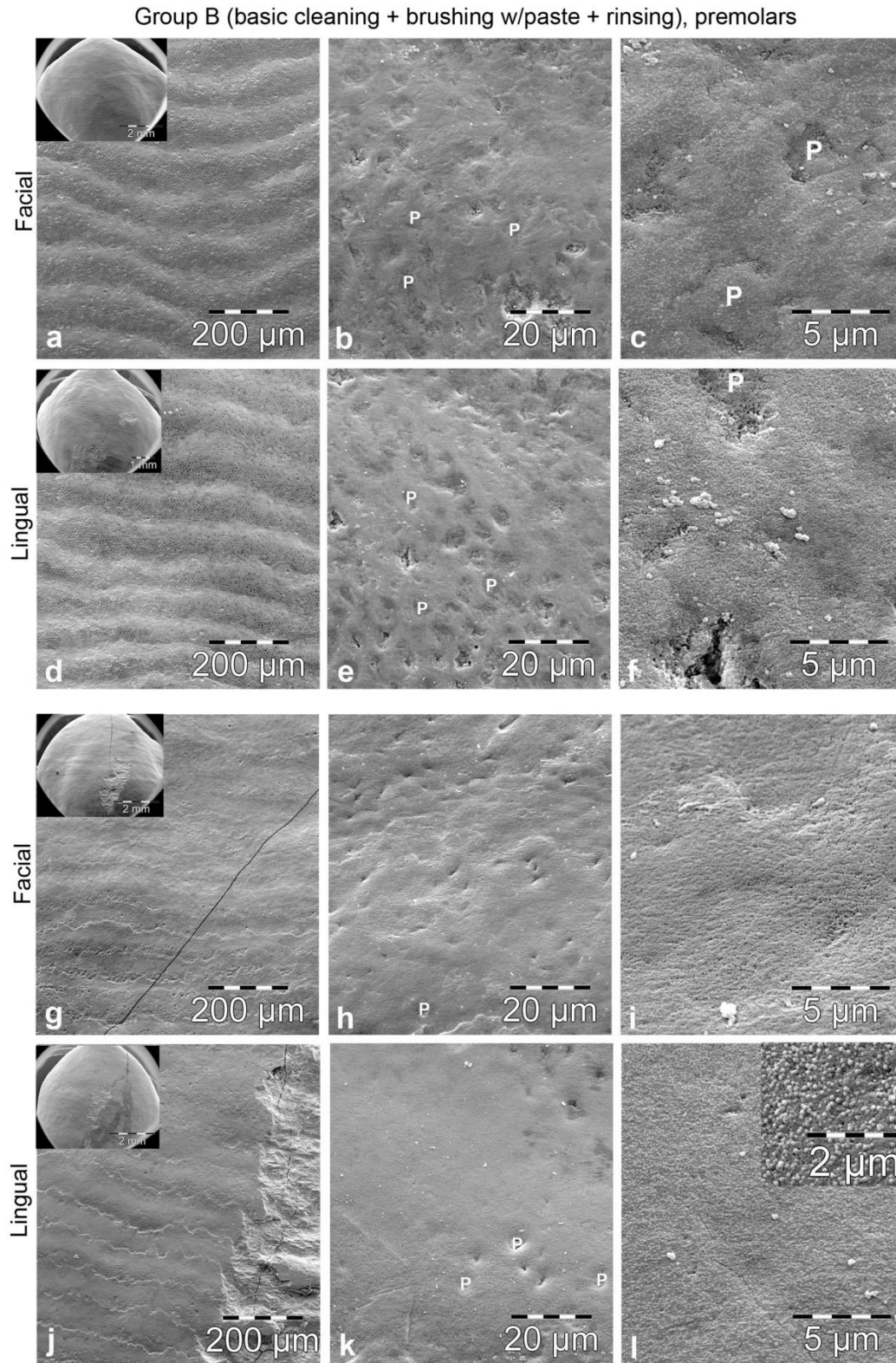


Figure 3



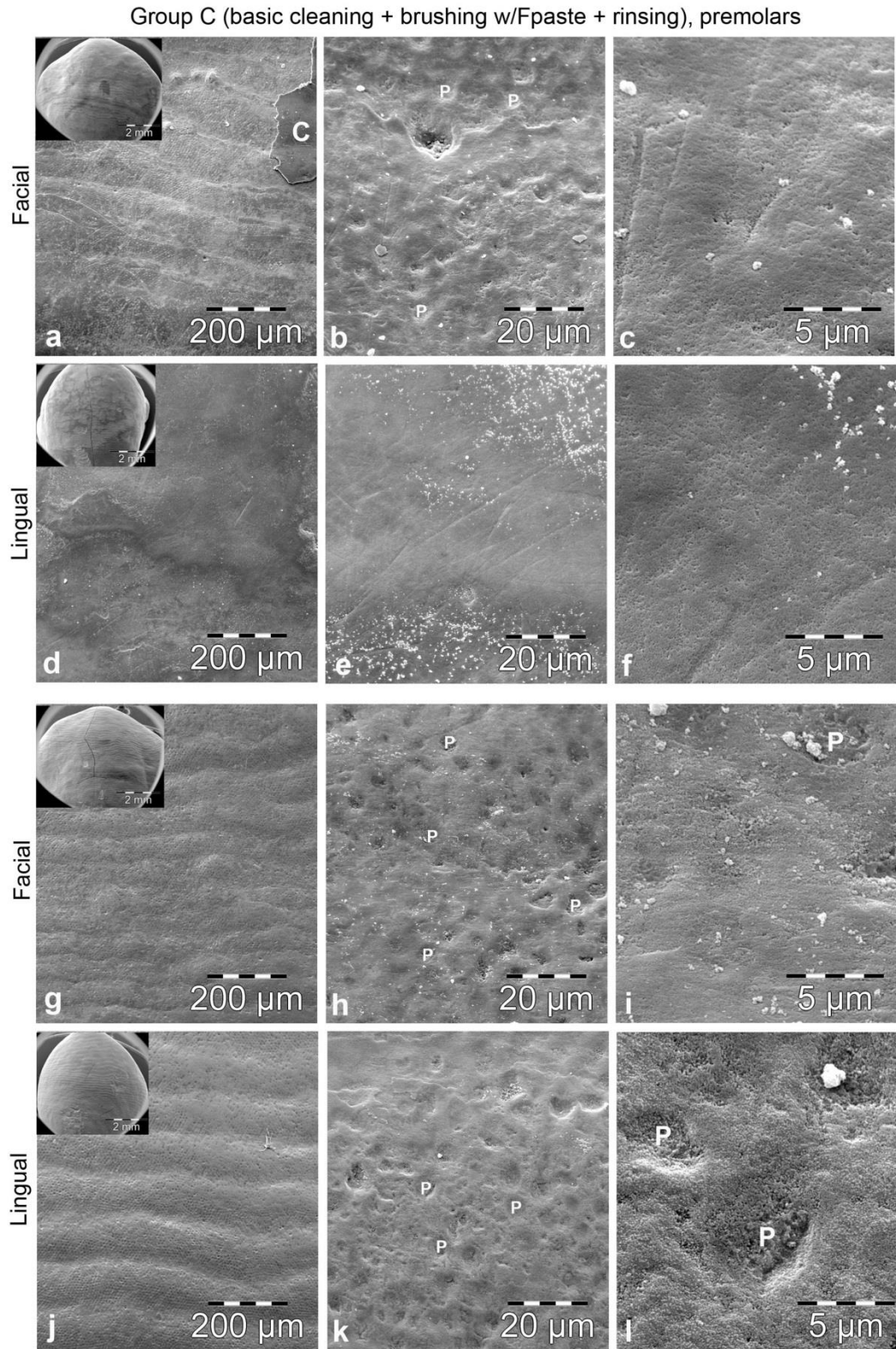


Figure 4



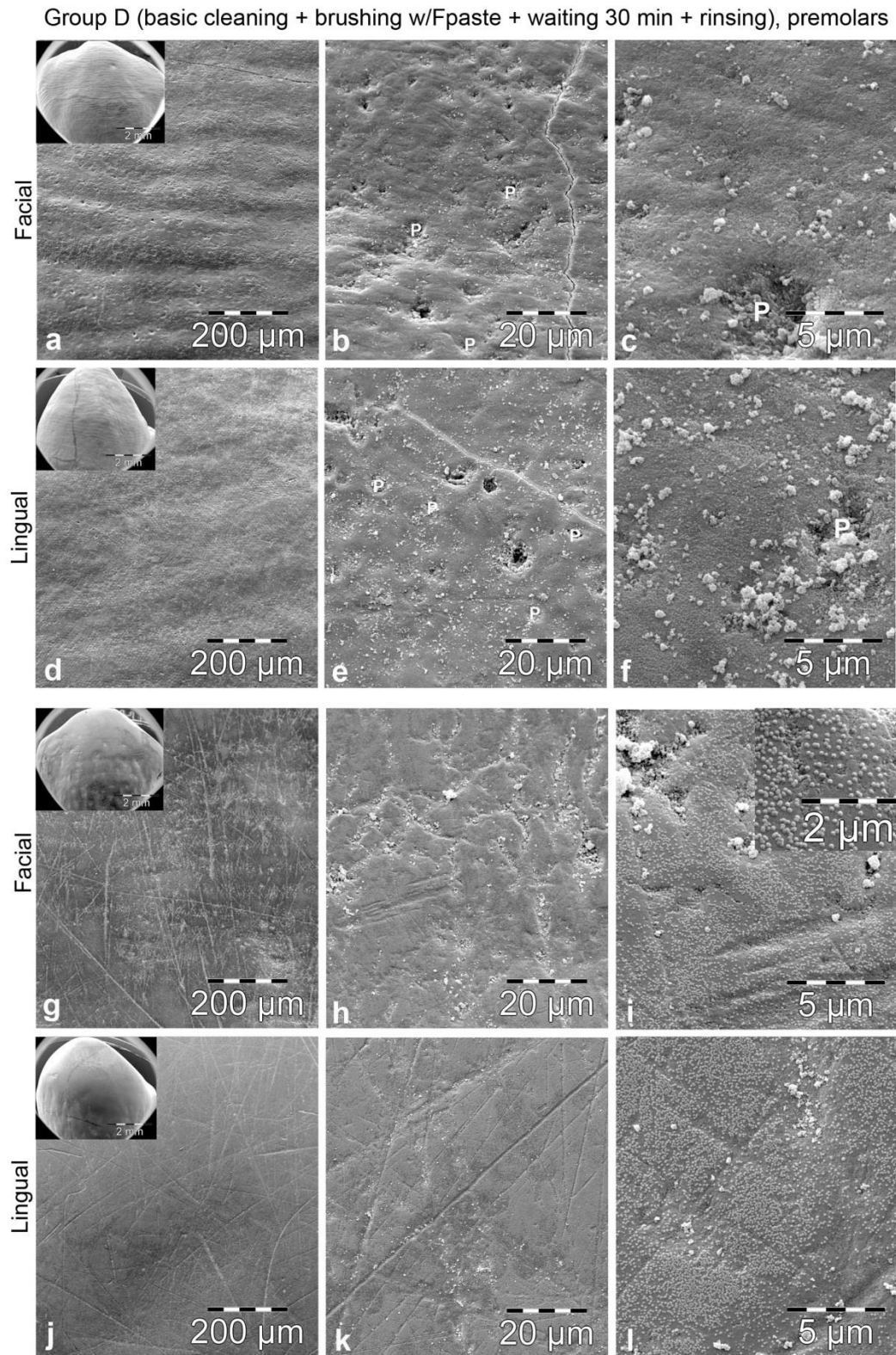


Figure 5



Group E (basic cleaning + etching), premolars

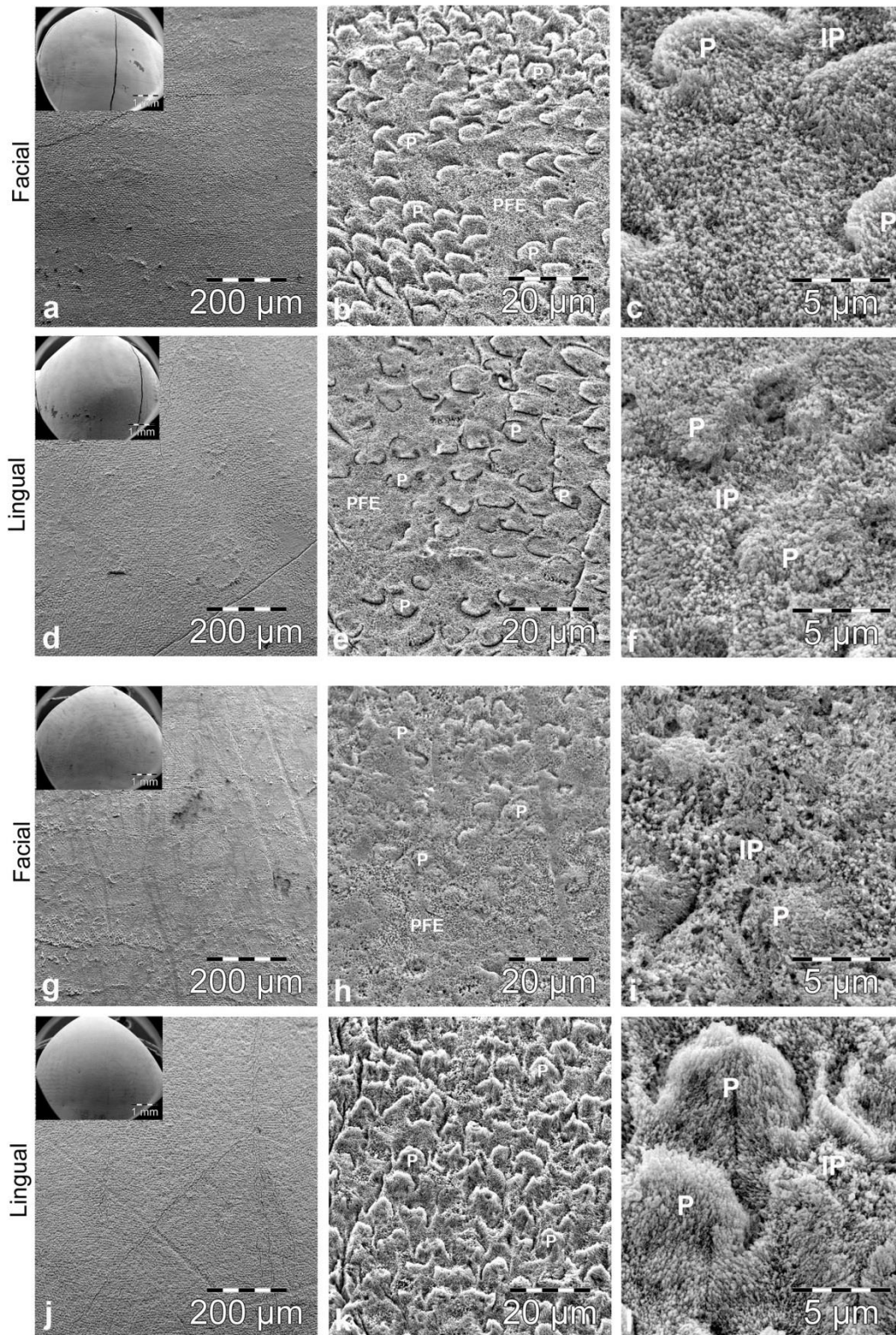


Figure 6



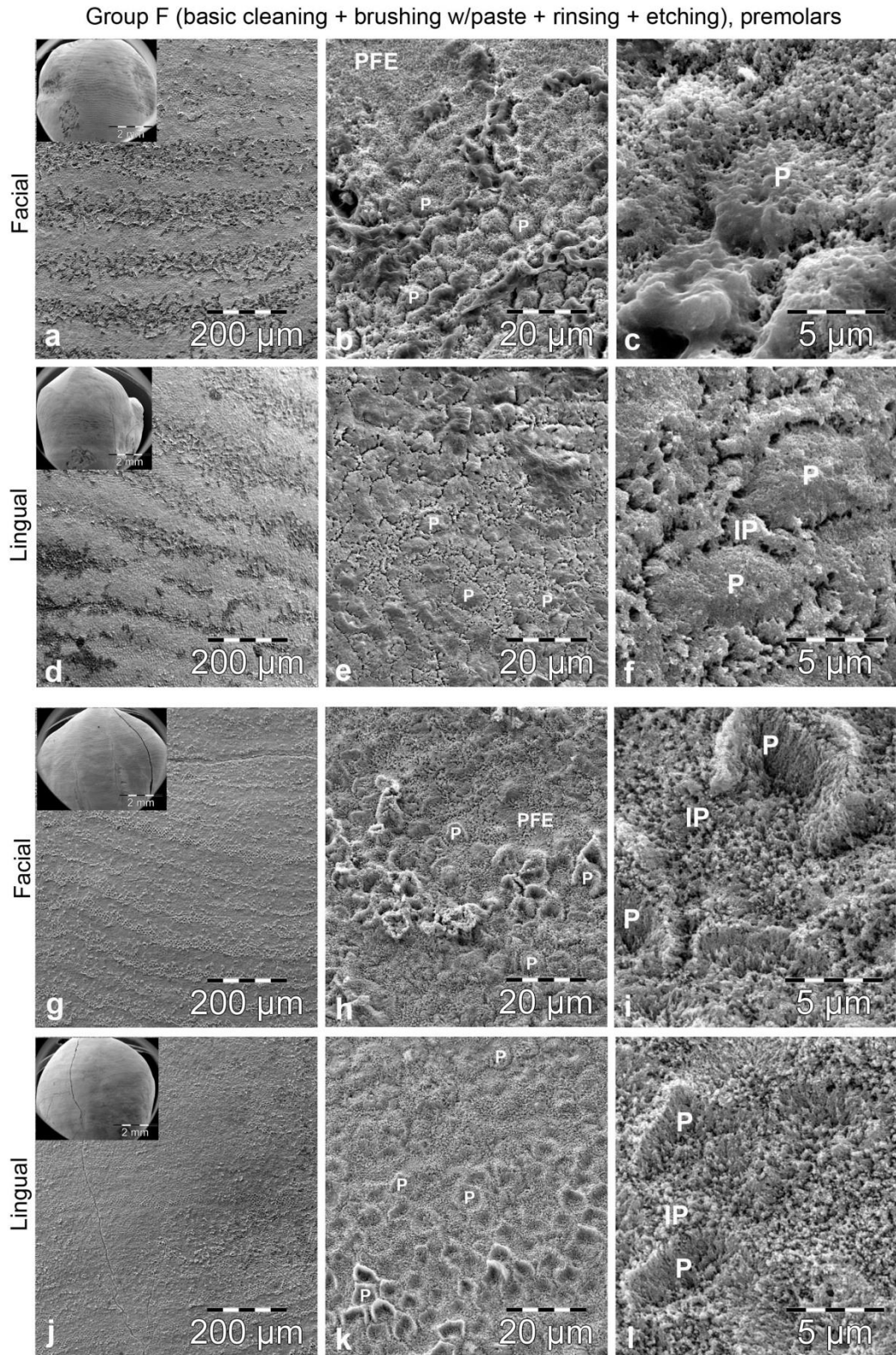


Figure 7



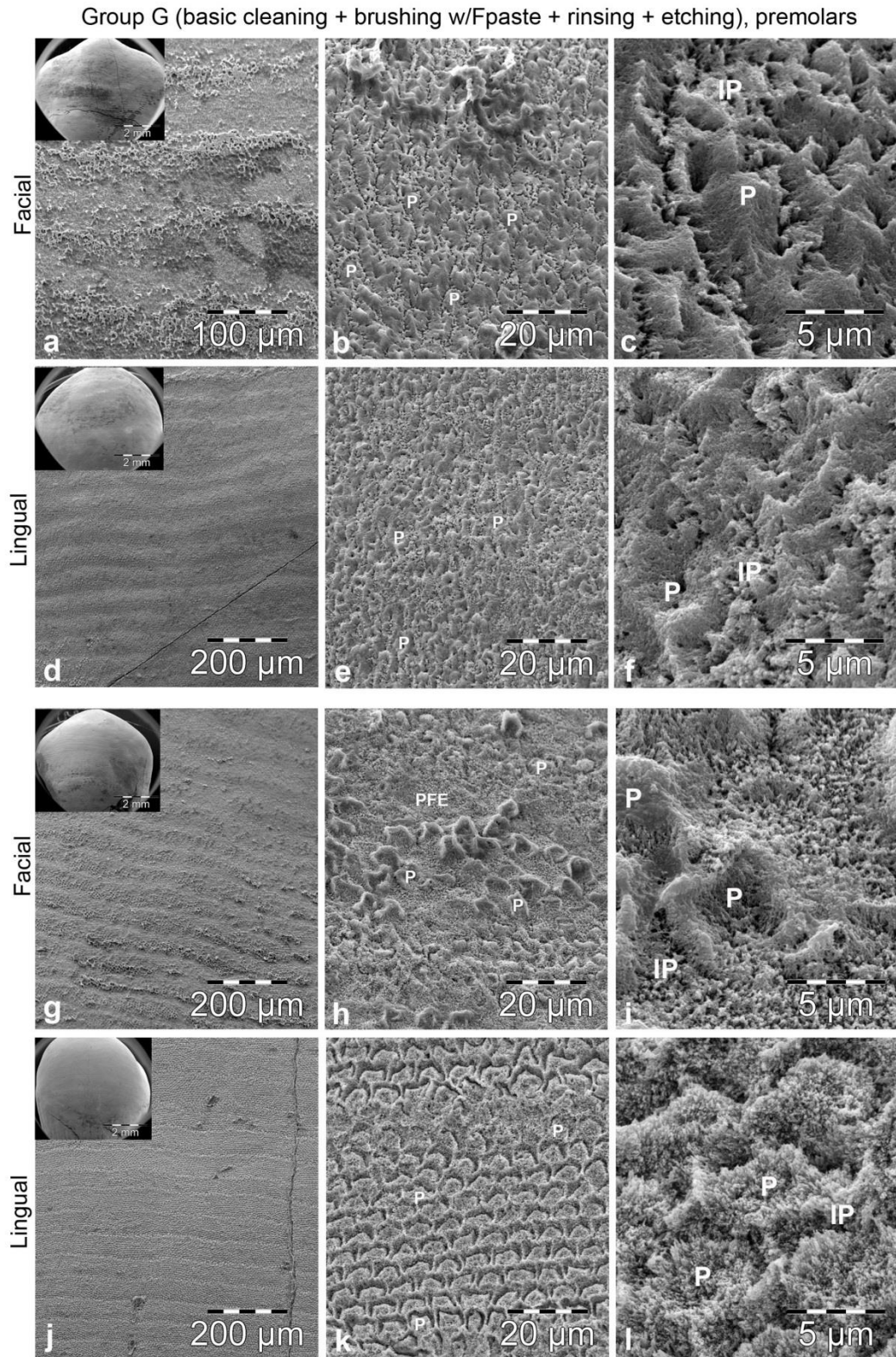


Figure 8



Group H (basic cleaning + brushing w/Fpaste + waiting 30 min + rinsing + etching),  
premolars

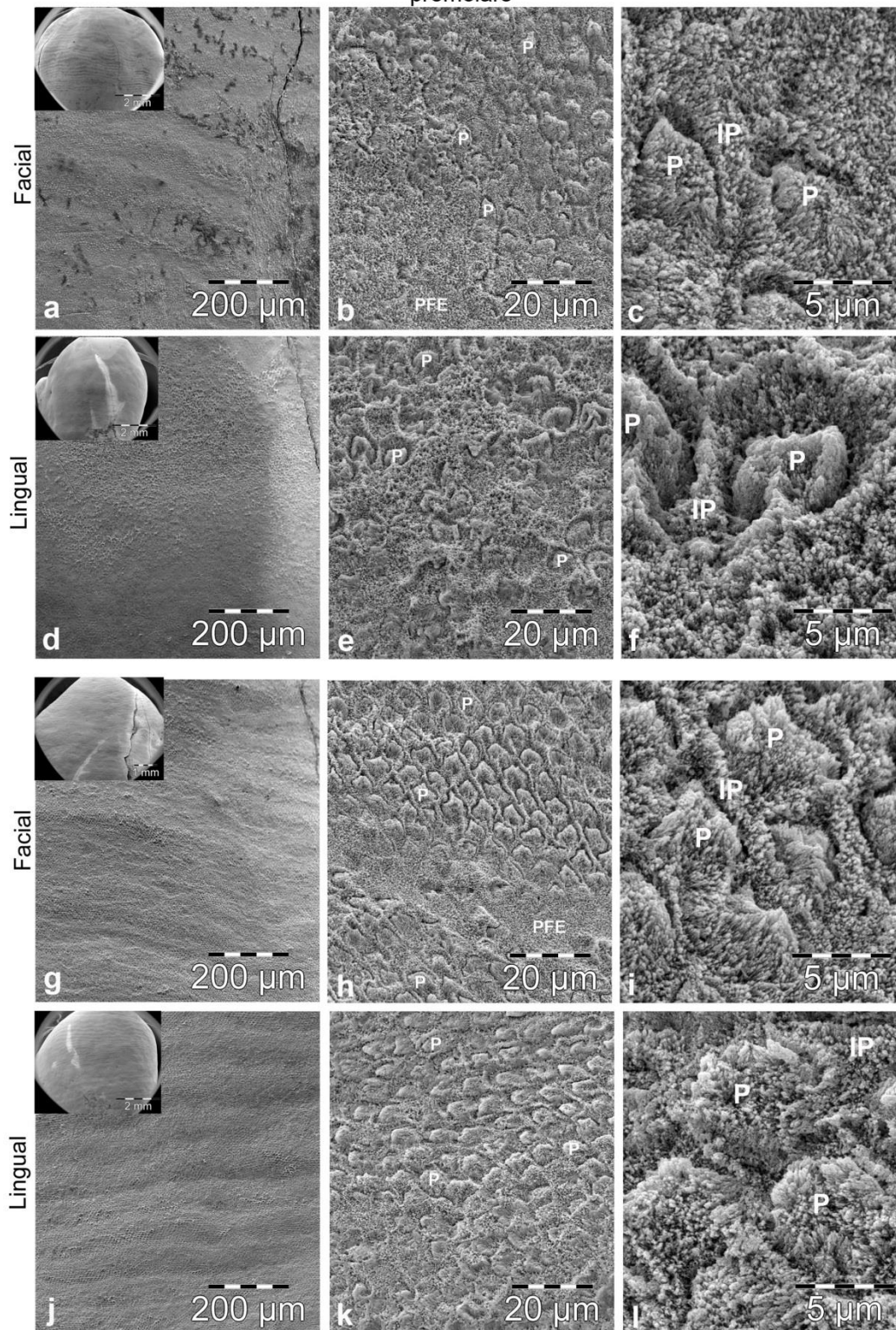


Figure 9



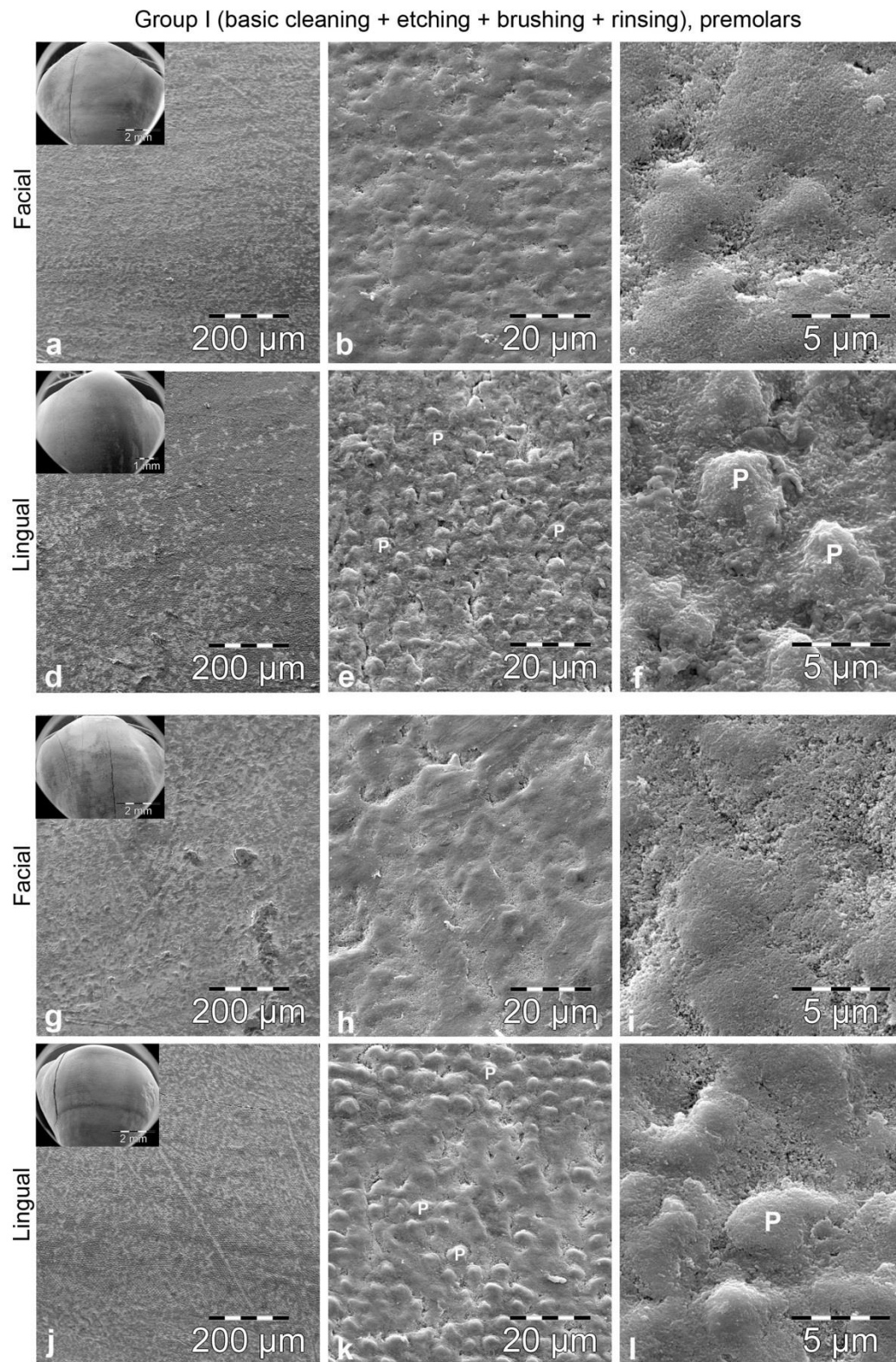


Figure 10



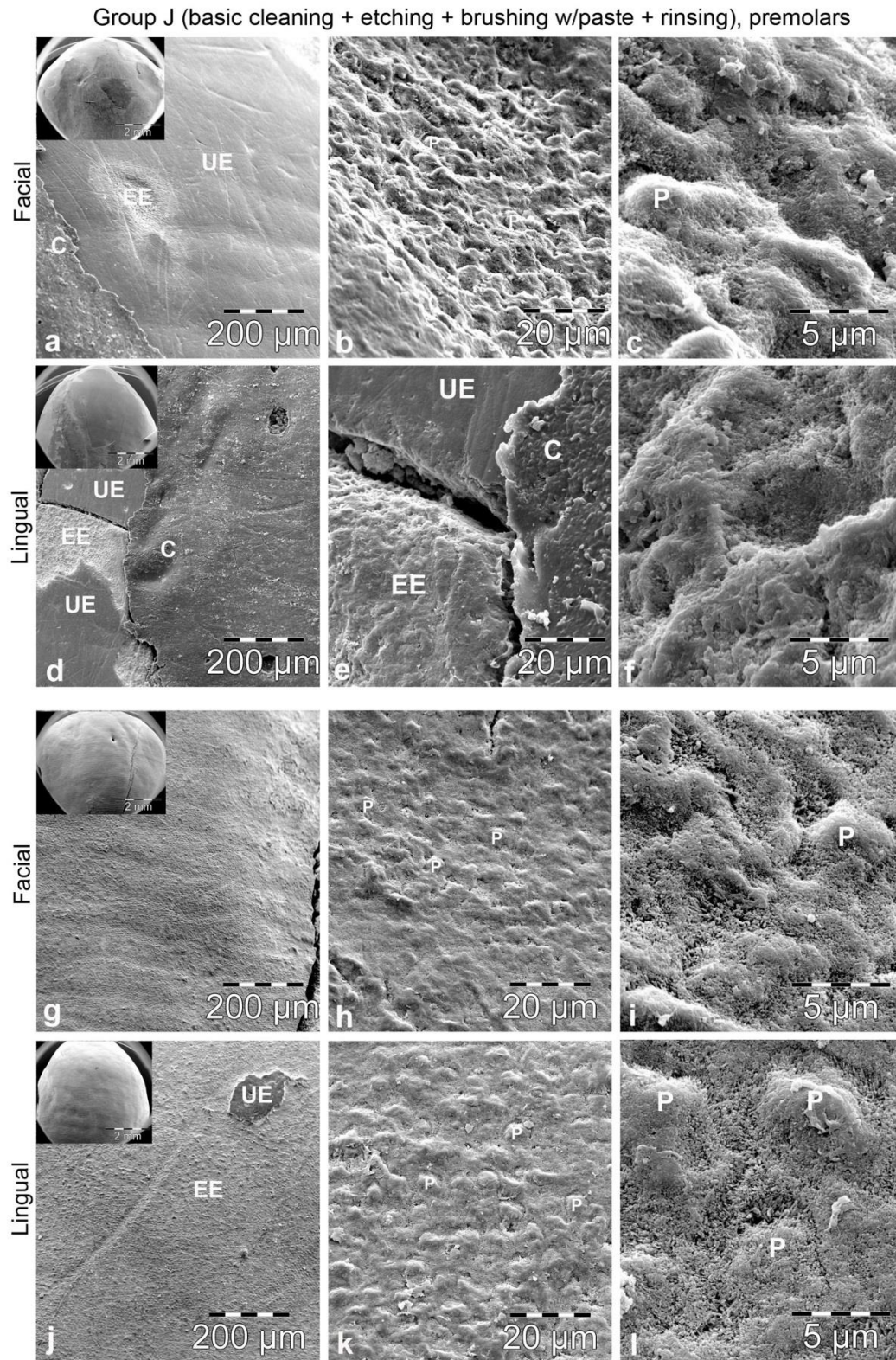


Figure 11



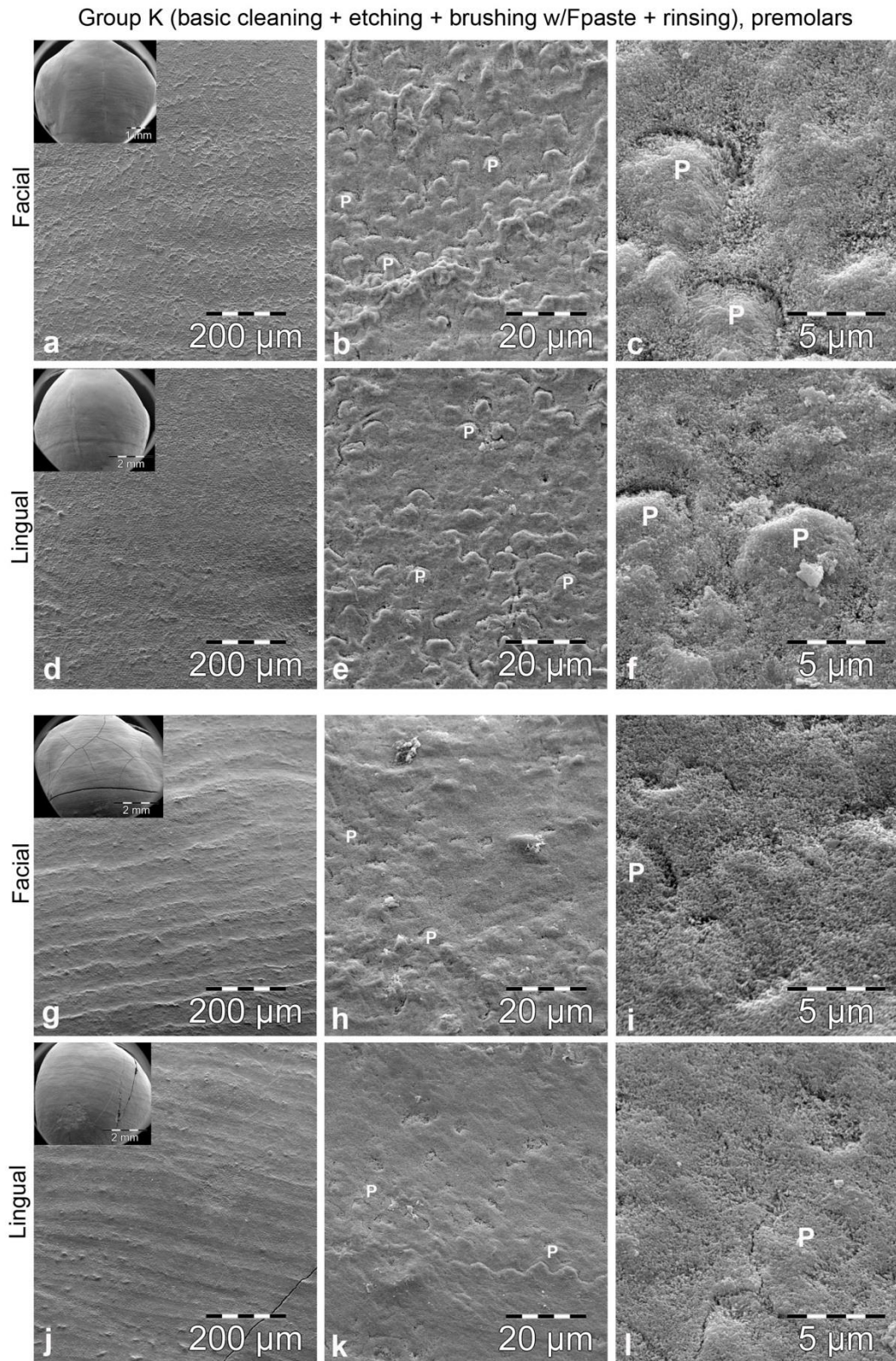


Figure 12



Group L (basic cleaning + etching + brushing w/Fpaste + waiting 30 min + rinsing),  
premolars

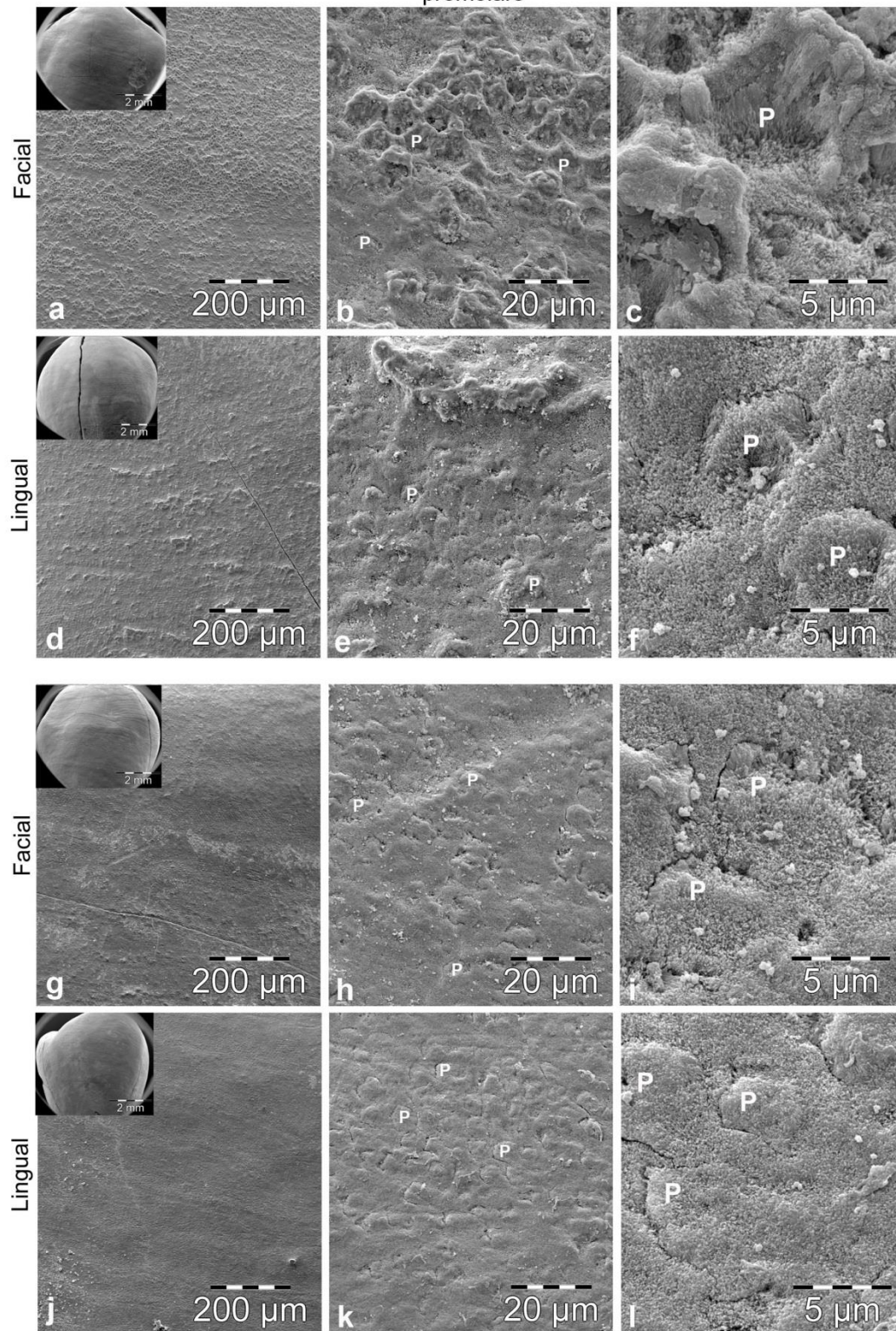


Figure 13



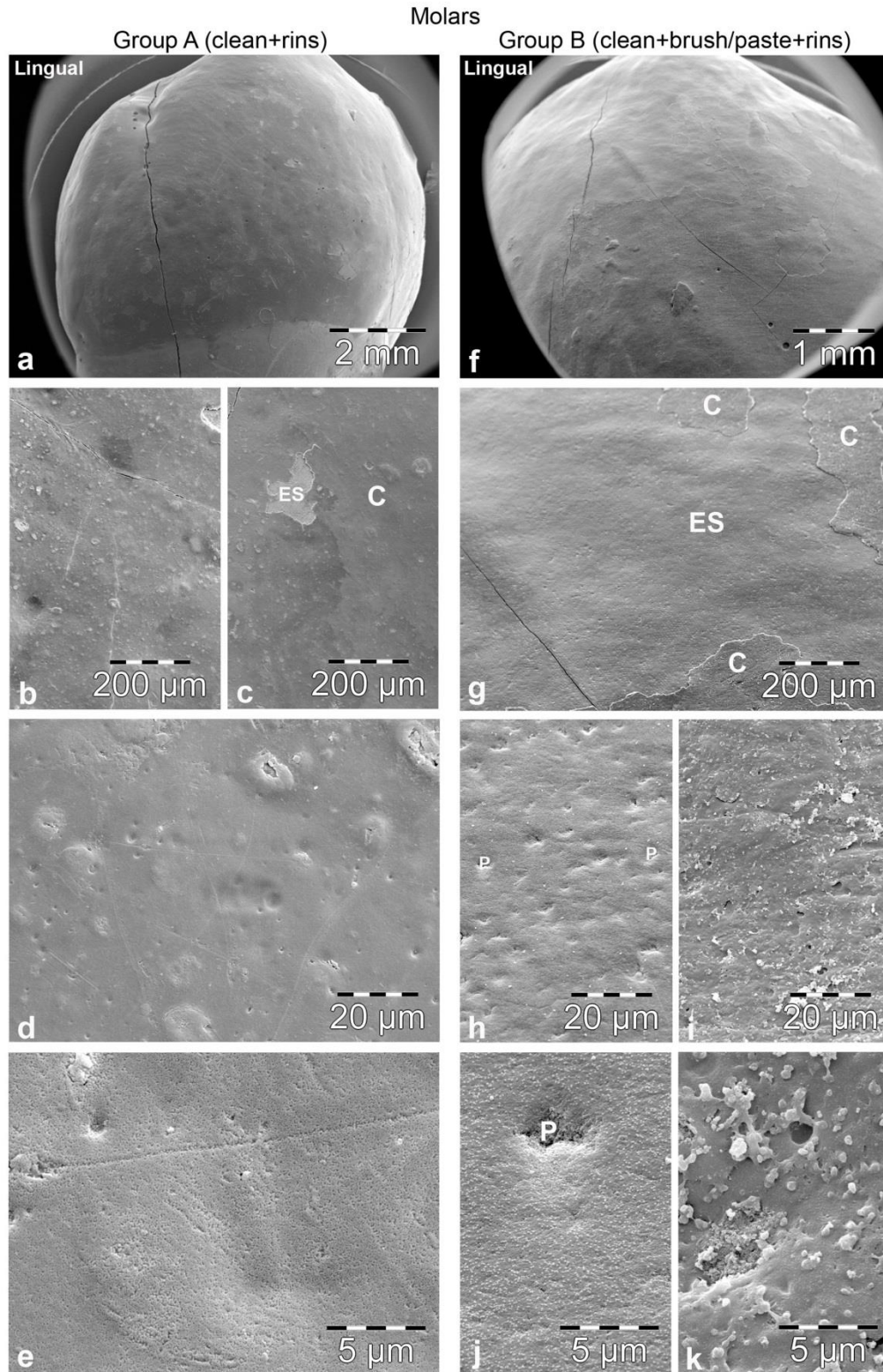


Figure 14



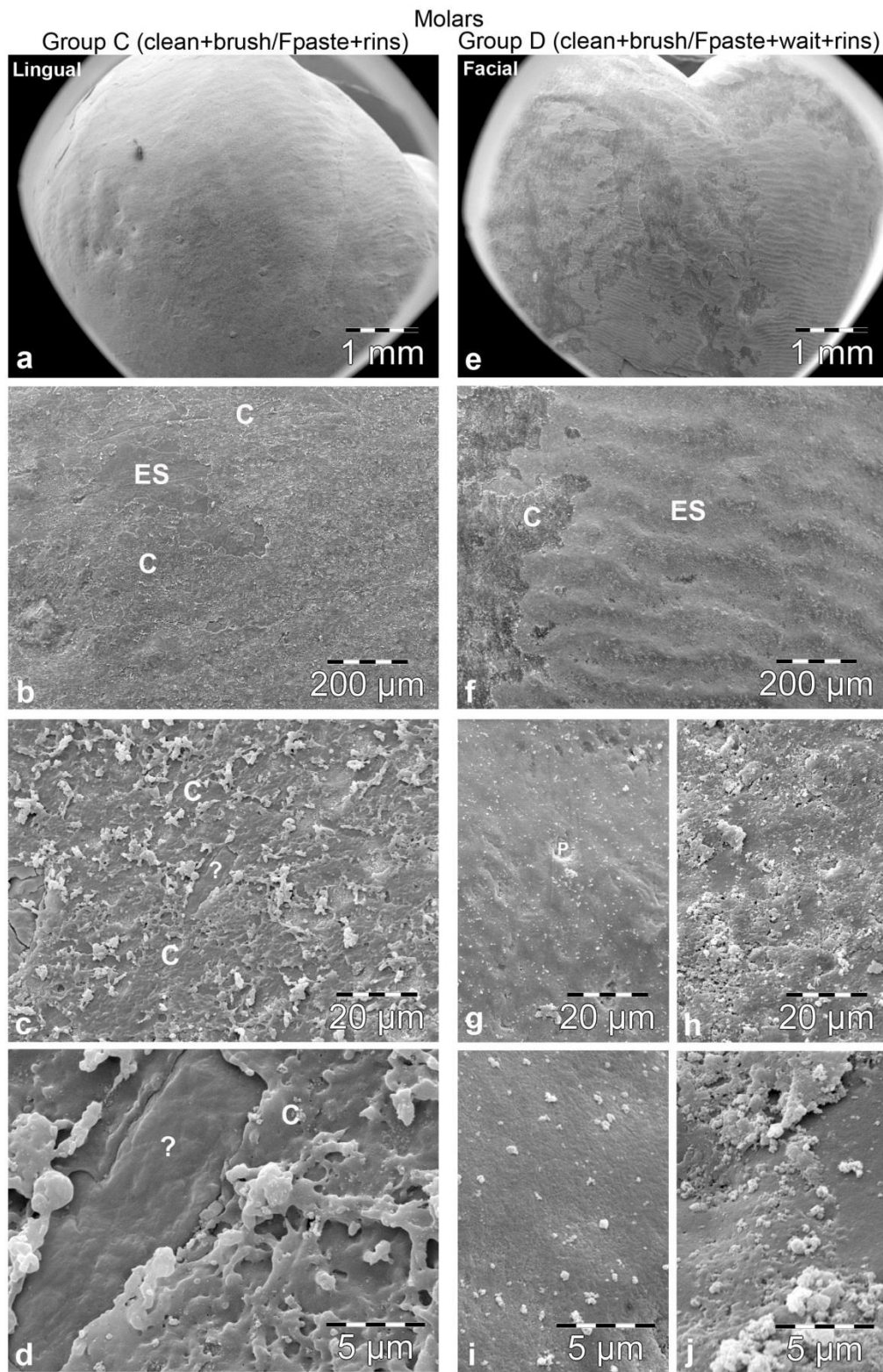


Figure 15

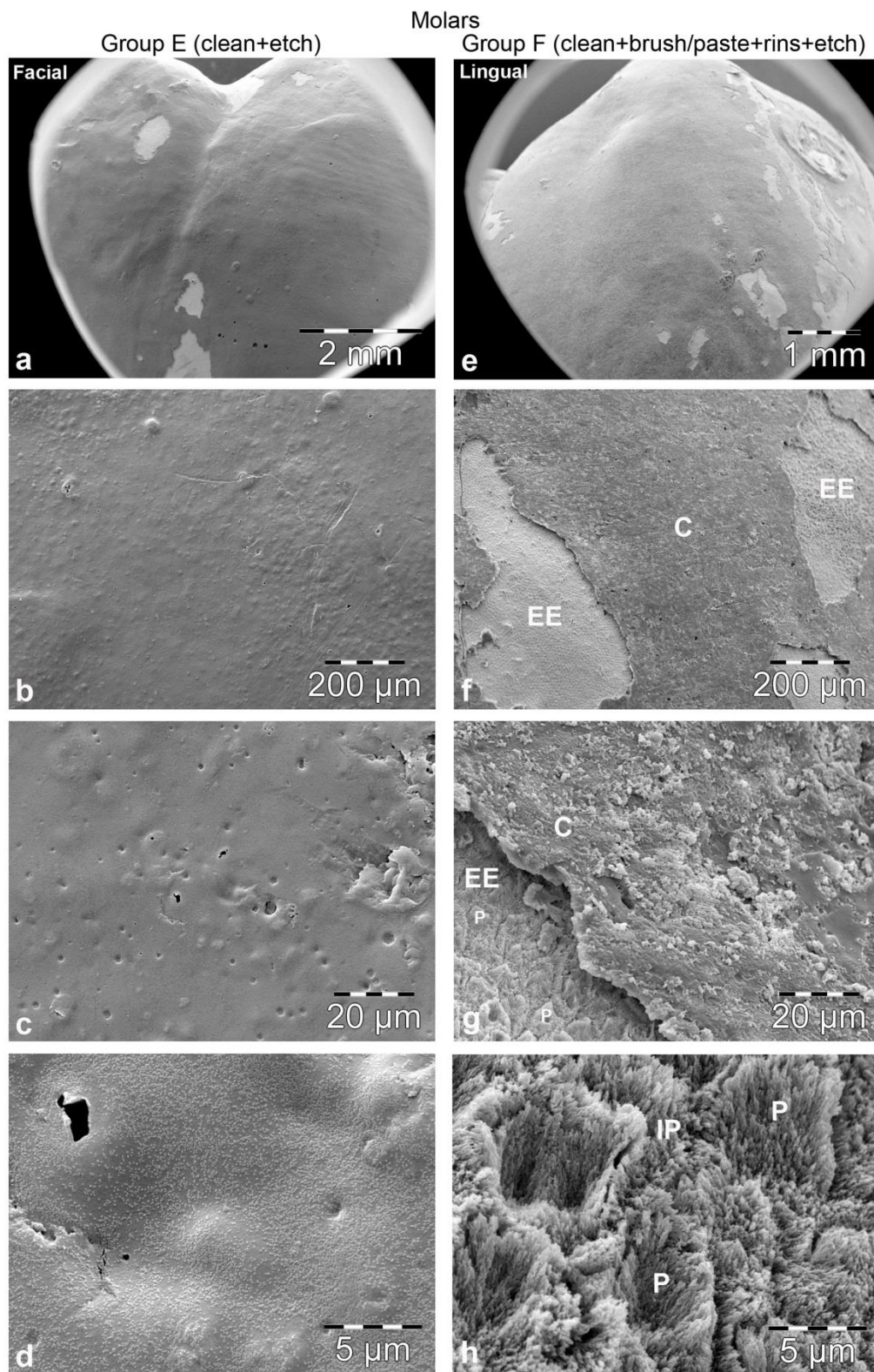


Figure 16



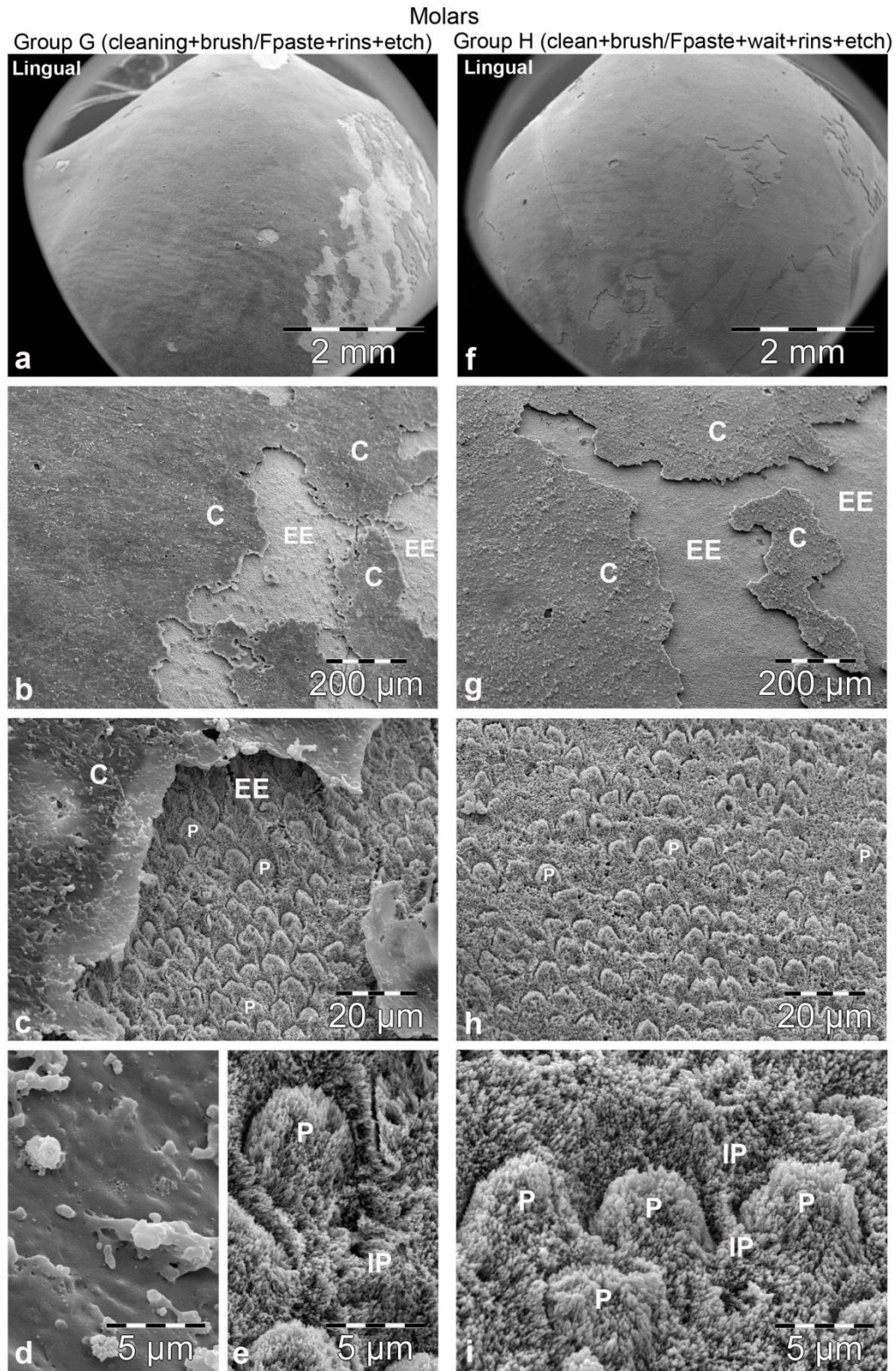


Figure 17

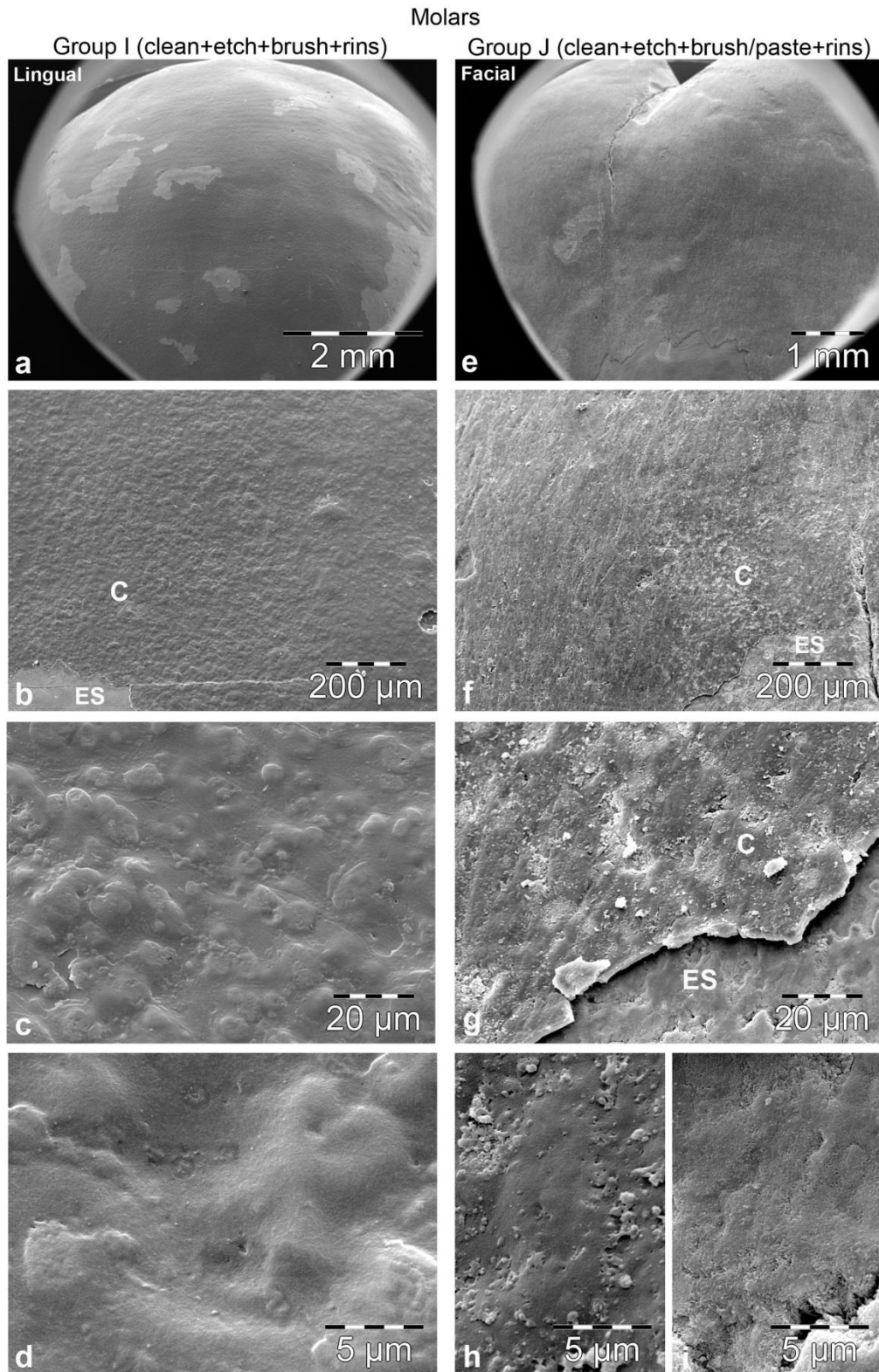


Figure 18



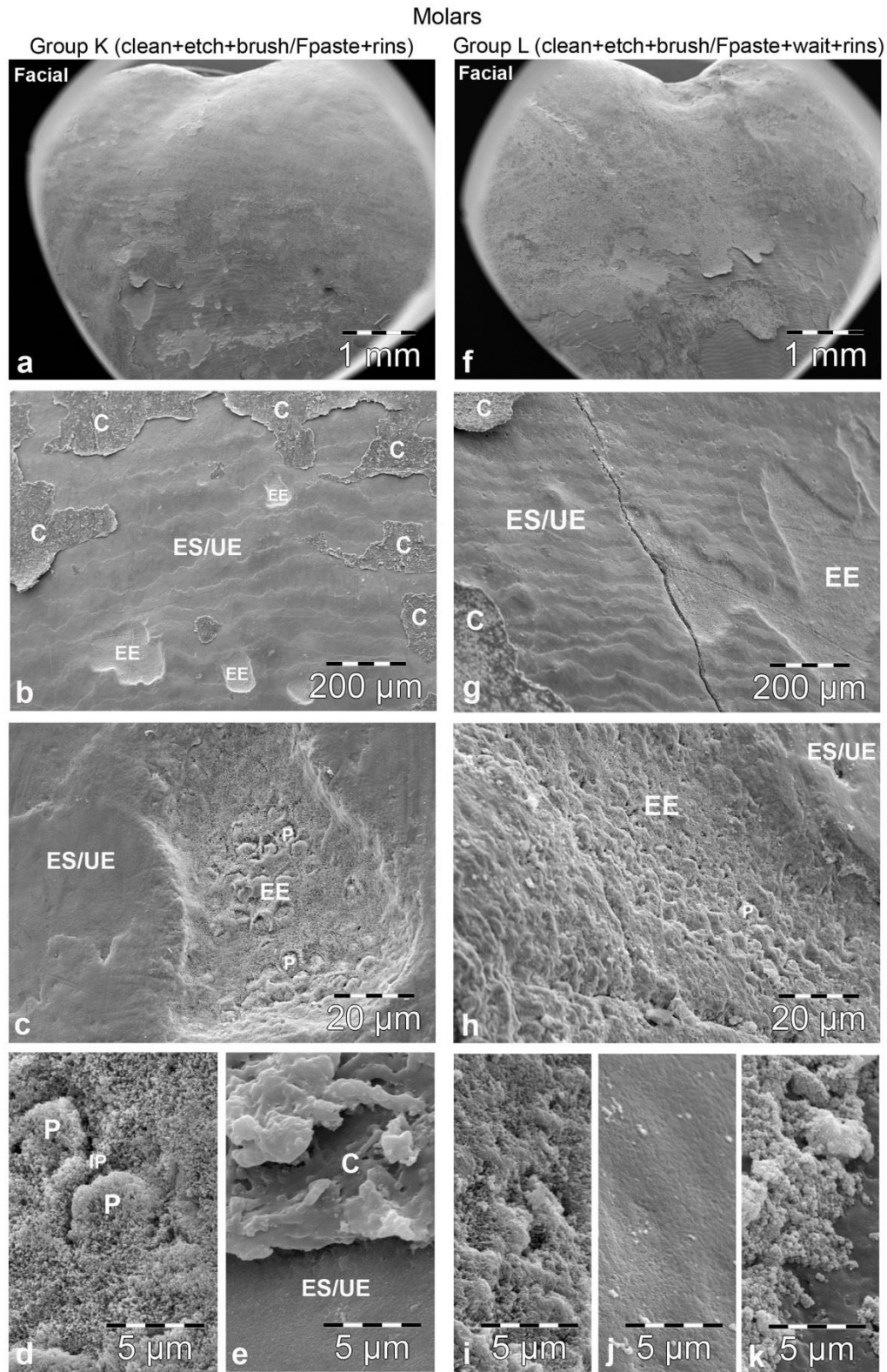


Figure 19

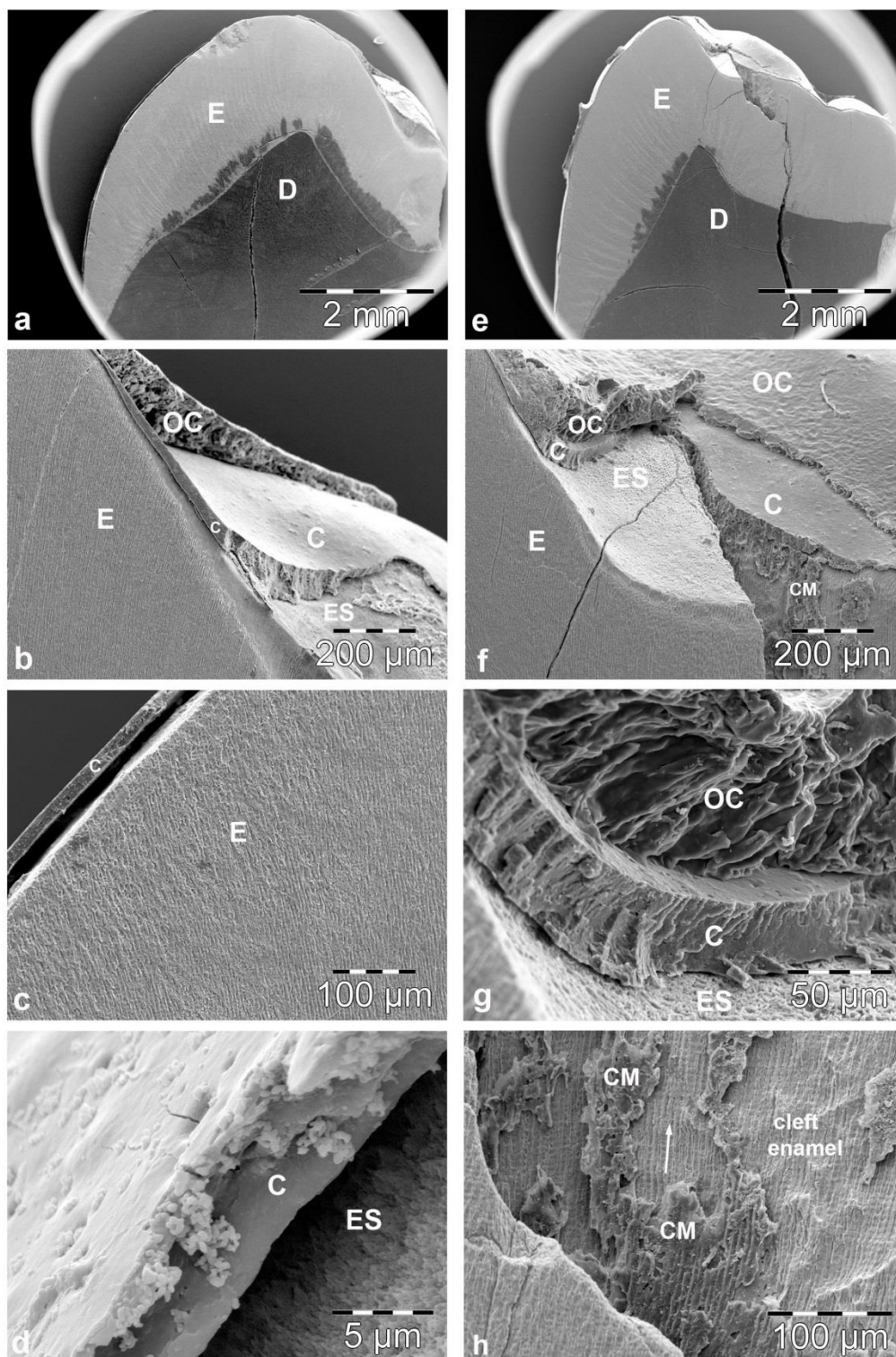


Figure 20



## Discussion

### Choice of teeth

The teeth were selected from a collection of extracted teeth at the Department of Oral Biology, Faculty of Dentistry, University of Oslo. This source provided an immediate access to a large material. It may be argued that a material of newly extracted teeth with a known history and a clinically more genuine enamel surface would have been preferable, but this was difficult to obtain within the scope of the present study. It was decided to use both maxillary third molars and first premolars; these teeth often have a relatively short exposure time to the oral environment since they may be extracted at an early age for reasons of prevention or convenience (third molars) or for orthodontic reasons (first premolars). Another possibility would have been to use bovine teeth (2). However, there are differences in structure and in behaviour towards clinically related procedures between human and bovine enamel (2). Since the experimental procedures in the present study are of a clinical nature, we decided to use human teeth.

The results of the present study indicate that the surface condition of the teeth prior to the experimental procedures of brushing and etching was variable with respect to wear, distinctness of perikymate, distinctness of prism ends, distribution of prismfree enamel, and presence of a surface coating. The teeth included in the present study were selected based on macroscopic observation, the criteria being clean and unworn facial and lingual aspects. In hindsight it would admittedly have been advantageous to view the specimens under a dissecting microscope during the selection of the teeth for a better evaluation of the surface condition prior to the treatments.

### The effect of brushing, etching and fluoride toothpaste

No obvious effects on the enamel surface were detected after brushing treatment alone, neither without toothpaste (Fig. 2) (8), nor with toothpaste, irrespective if it contained fluoride (Figs. 4, 5) or not (Fig. 3). However, this is difficult to evaluate since the condition of the enamel surfaces at a microscopic level prior to the



treatments is unknown. Eisenburger et al. (8) found that there was no difference in enamel wear after 12 brushing strokes compared to 500. Although this was a non-significant trend, the finding indicates that one minute of brushing, as in our study, would have been sufficient for detecting any possible effects on the enamel surface.

Brushing with toothpaste left what is assumed to be remnants of toothpaste on the surface, in the form of a grainy-fluffy material (Figs. 3-5). This material was lost when brushing was followed by etching (Figs. 7-9), being removed together with a superficial layer of enamel. Much less toothpaste residue was left after brushing an already etched surface (Figs. 11-13). This is somewhat surprising since one would think that the material is more prone to remain on a rough surface than on a smooth surface. More material remained when the toothpaste was left on the surface for 30 minutes before rinsing (Figs. 5, 13). On the molars a fluffy-grainy material was more prominent on the coating than on the enamel surface proper (Figs. 15-18). However, it is possible that some of this material stems from the coating itself since it was also seen on teeth brushed without toothpaste (Fig. 14i,k).

A deposit consisting of relatively evenly distributed spheres with a diameter of about 0.1  $\mu\text{m}$  was observed on some enamel surfaces brushed with toothpaste, both on premolars (Figs. 3c,f,l, 4l, 5f,l,j) and molars (Fig. 14j). It was even observed on the coating of a molar that had not been brushed with toothpaste (Fig. 16d). The character of this material and the reason for its variable presence is unclear.

Poole and Johnson (7) have described how different acids affect the human enamel surface. There seems to be no consensus as to which acid to use when studying the enamel surface. Different types of studies have chosen different types of acids, e.g. hydrochloric acid (6), citric acid (8) and phosphoric acid (9). We chose nitric acid since this has a well-documented effect on the enamel e.g. Li and Risnes (10).

Etching the enamel surfaces with 0.5 % nitric acid for one minute revealed the typical enamel structure with prisms, interprism, prism sheaths and areas with

prism-free enamel (Fig. 6). No obvious modulating effect of brushing with toothpaste on the effect of subsequent etching was detected, irrespective if the toothpaste contained fluoride (Figs. 8 and 9) or not (Fig. 7). The present method did not allow an evaluation of the amount of material dissolved by the etching, which could have indicated differences in crystal solubility.

Fluoride studies have used different methods for measuring loss of enamel quantitatively, such as profilometry and microhardness testing (11). Several authors seem to agree that topical fluoride application have a protective effect by hardening the surface and that fluoride toothpaste reduces erosion and abrasion of enamel (12,13). The immediate effect of simultaneous fluoride application and mechanical brushing, as in our design, is less evident.

A very obvious modulating effect of brushing on etched enamel was observed. The superficial enamel seemed to have been “softened” by the acid. The enamel had been partly removed and smeared over the surface, blurring the identity of the prisms. Similar findings concluded that “...softened enamel is highly unstable and potentially easily removed by short and relatively gentle physical action.” (8). This observation has been found on presoftened enamel compared to normal enamel in vitro (14). In our study, there might be a slight difference between the effect of fluoride toothpaste (Figs. 12, 13) compared to no toothpaste (Fig. 10) and nonfluoride toothpaste (Fig. 11), the individual crystals appearing more distinct and tightly packed in the former. Thus, it may be speculated that the available fluoride to a certain degree had promoted some crystal growth on a surface from which calcium and/or hydroxyapatite already had been chemically and mechanically mobilized.

The fluffy-grainy material observed was found in both fluoride and nonfluoride groups. Therefore this is likely to be remnants of toothpaste. Studies where  $\text{CaF}_2$  globules have been found have mainly used high concentration fluoride and/or acidified fluoride gel (15, 16).  $\text{CaF}_2$  is also shown to be more soluble in water versus saliva (17). As the last step in our study the treated specimens were rinsed with water and this may have influenced the results. There is stronger evidence of fluoride’s protective effect in studies using high concentration

fluoride solution (16). In our study we used toothpaste with 1450 ppm and therefore a possible effect may have been difficult to detect. In addition we only had one application of fluoride toothpaste, whereas it seems as the effect of fluoride may be more profound with several applications over time (12).

### **The nature and origin of the observed coating**

All the specimens were initially cleaned by brushing with dishwasher detergent and water in order to remove possible deposits acquired after extraction. Other studies have used pumice or EDTA as pretreatment to SEM imaging to remove smear layer (3). As we were studying the enamel surface we had to use a gentle pretreatment to avoid destroying the surface structure. EDTA would have removed the original enamel surface and the pumice would have caused unwanted abrasion. Our gentler treatment evidently was not sufficient to remove the observed coating on the molars and one of the premolars. Since the coating seemed unaffected by acid, it was assumed to be organic in nature. And since the teeth used in the present study had been kept in 70 % alcohol for a period of time, the coating was fixated, i.e. denaturated. This may account for its relative sturdiness during brushing, allowing its identification and observation. One would assume that the covering would have been removed more readily in its fresh, unfixated state in the mouth, although the pellicle has a certain mechanical resistance also in vivo (14).

The coating was partly removed by brushing. It evidently protected the enamel from the etching effect of acid, since coating removed subsequent to etching revealed unetched areas of coating-free enamel surfaces.

We distinguished two different coating layers: a general coating covering the whole enamel surface and a coating covering only the occlusal aspect (Fig. 20). Although probably of organic nature, the origin of the coatings is unclear. The difference in occurrence between molars and premolars may indicate a partly origin from the enamel organ since the third molars included in the study probably had been exposed to the oral environment for a considerably shorter time than the premolars and may have been difficultly accessible by toothbrush.

The thickness of the coating, 10-40  $\mu\text{m}$ , exceeds the thickness reported for the acquired pellicle alone (4). Thus, the general coating may represent a combination of a primary and an acquired cuticle/pellicle (4) with main contributions from saliva. The acquired pellicle is formed as soon as the tooth erupts (5).

It was interesting to observe that the general coating was continuous with material observed in an enamel cleft (Fig. 20f,h). Since the time of formation of the cleft is unknown, the origin of its content is uncertain. The fact that it shows impressions of longitudinally oriented prisms is not decisive for its time of origin since this configuration may be in accordance with both an odontogenic and a postodontogenic and even posteruptive origin. The second coating on the occlusal aspect is more difficult to fathom, especially since it is formed externally and hence after the general coating. It could possibly represent a part of the enamel organ being lodged in the cavity of the molar occlusal aspect at eruption. Meckel (4) described the cuticle from a fully impacted and surgically removed upper premolar viewed in an electron microscope. He found the thickness of the cuticle to range between 1- 8  $\mu$  (4). A plaque contribution to the observed occlusal coating can not be excluded.

## Conclusions

No obvious effect on the enamel surface was detected after the brushing treatment alone, with or without toothpaste. Brushing with toothpaste, with or without fluoride gave no additional effect other than leaving a fluffy-grainy material on the surface. A very obvious modulating effect of brushing on etched enamel was observed. After brushing with fluoride paste individual crystals tended to be more obvious compared to brushing without paste and brushing with nonfluoridated paste. The sample size is not large enough to conclude any further with regards to this subject. Brushing tended to remove the coating present on the molars. The coating protected the enamel from the etching effect of acid, since coating removed subsequent to etching revealed unetched areas of

coating-free enamel surfaces. The nature of the coating observed on molars is not clear, but it is suggested that it may in part be of an odontogenic origin.

## References to part two

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